

What future for *perestroika*?

The general recognition that Mr Mikhail Gorbachev may be skating on thin ice has caused widespread dismay, but the future is not nearly as bleak as it seems. Adventurous decisions now could assure the future.

MR MIKHAIL Gorbachev is such an appealing character that even people outside the Soviet Union have begun worrying that he may not be able to keep his jobs as General Secretary of the Communist Party and as President. But the anxiety does not spring from pure fondness. People elsewhere regard Gorbachev as the best assurance that the Soviet Union will become a more welcome neighbour than in the past, not to mention the prospect that his good intentions should also benefit the Soviet people. The trouble is that many of the dreams he has encouraged are not coming true. People may have won from *glasnost* a marvellous sense of freedom, but after five years the city shops are still empty. Dr Andrei Sakharov expressed the worry more generally in London two weeks ago (see *Nature* 339, 646; 29 June 1989) when he said that even those who considered themselves "well-informed" had been shocked to learn at last month's congress how impoverished the Soviet Union has become.

The essence of the difficulty is widely understood. First, Soviet agriculture has been a notorious failure since long before Gorbachev's time, but its product has been only marginally improved by the decision that profit-orientated cooperatives should be free to sell their produce for what the market will bear. Second, Soviet exports consist mostly of commodities such as gold, oil, cotton and wood which do not reflect either the sophistication of the Soviet Union's potential in manufacturing or the sophistication of its need of imports. Third, the domestic manufacture and distribution of the familiar needs of everyday life is made uneconomic by a complicated system of planning, subsidy and price control, but hopes that the system might be made efficient by trading subsidies for increased salaries disappeared when last year's special party conference ended.

Gorbachev cannot do much about the last of these problems without running headlong into the underlying difficulty of his position — that his licence for *perestroika* carries the condition that the political foundations of the Soviet state should not be moved. Exhortation may help, as may the new Supreme Soviet, but the prospects of speedy change are not bright. But the export business and agriculture are surely more tractable problems where something could be done.

The irony of the Soviet Union's position in the products of advanced technology, which are the only means of earning a sophisticated return in international trade, is

that it appears to be capable of competing successfully with the West in at least one field — the design and manufacture of weapons of various kinds. But the military enterprise is isolated from indecision and inefficiency in the Soviet Union within a kind of economic enclave.

So why not follow that example with sectors of the Soviet industrial empire likely to be successful in the export business? Mr George Sores, the New York financier of Hungarian extraction turned friend of the Soviet Union, has recently been urging that the Soviet Union should follow China in setting up geographically defined economic zones free from domestic restrictions. One snag is that free economic zones would depend on capital and ideas from outside, which would not readily be forthcoming while the conditions for investment in the Soviet Union remain ambiguous. Another is that even quarantined zones such as these depend on the efficiency of the hinterland.

But why not create functional enclaves instead? The Soviet Academy of Sciences, for example, is collectively a successful designer and manufacturer of instruments and technical equipment. The Institute of Nuclear Physics at Novosibirsk has a modestly successful business in electron accelerators of various shapes and sizes, and the instrument institute at Leningrad in which the academy is a partner (the others are ministries) is awash with ideas but has not yet made a mark outside the Soviet Union. Why not give organizations such as this not just the freedom but the responsibility of operating internationally, preferably in concert with partners elsewhere?

The problem of agriculture is more arcane. As farmers (and taxpayers) elsewhere are well aware, the application of technology to farming is now so well practised that the chief difficulty is avoiding surpluses. It really is disgraceful that the Soviet Union finds the trick elusive. Gorbachev appears to have diagnosed the problem correctly, and has established the principle that farmers should be given leases on manageable blocks of land for 50 years or so. But last weekend, Mr Igor Ligachov, a member of the Politburo who is chairman of the government commission on agriculture, argued that peasants did not wish to leave their collective farms, which are the backbone of Soviet agriculture. Although Ligachov's statement reads like dissent, both men are probably correct: today's peasants, with no tradition of independent farming, have every reason to shy from the responsibility



of independence. That is why the first need is for an agricultural extension service ready to hold the hands of newly independent farmers. The snag, of course, is that creating such institutions will take more time than Gorbachev can spare. □

Summit technology

Next week's summit in Paris should pay more attention than planned to the benefits of technology.

WHAT can the heads of seven governments (Britain, Canada, France, Italy, Japan, West Germany and the United States) usefully do to make the world a tidier place? They have one obvious task, that of plotting a course towards a more stable economic system, as the Organization of Economic Cooperation and Development (OECD) has been helpfully reminding them this week. Inflation, the bugbear ten years ago, is now again so much a threat that some observers have begun pleading that even a recession would be welcome. There are two obvious objections.

At least in the short run, a recession would impede the Bush administration from cutting the federal budget deficit in the United States, itself one of the engines of inflation internationally. And a general recession now would further damage two of the important but unrepresented interests at next week's meeting — the poor countries of the world would again be disappointed, while brave hopes now current that the future may be brought about benignly would be attenuated as governments cut back on innovation and improvement.

Economists will say that the summit meeting could take two useful steps to avoid recession while keeping inflation down — resolve on further steps towards monetary coordination and to the liberalization of international trade. The usual communique will no doubt offer the usual promises, but the summit governments could usefully try on this occasion to be more imaginative. When the same governments last met in France, in 1982, they were startled that President François Mitterrand should have made technology the centrepiece of the agenda. He then, like the rest of them then and now, was seeking to harness science and technology more directly to social benefit.

It would be a pity if the summit perpetually ignored the essence of the old Mitterrand argument. There are two simple limitations of the benefits that may flow from science and technology: the limited stock of learning and skill, and the tendency for it to be occupied (often at the behest of governments) on fruitless projects. This argues both for collaboration (which, in the market-driven West, means the mobility of people) and that choices should be made by the potential beneficiaries rather than by governments, which are not always the best proxies for them. The most serious threat to this opportunity is the tendency for governments to regard technology as an instrument of national chauvinism. It is time they stopped. □

Revolution in France

Whatever the failures of the revolution 200 years ago, it seems to have left French science unscathed.

NEXT Friday's Bastille Day will be the second centenary of the French Revolution, but will also see the opening in Paris of this year's summit meeting between the heads of state of the seven major industrialized countries of the West. The coincidence is not accidental. At some early stage in the planning of the summit, President François Mitterrand was evidently keen that others should not forget the glorious history of France. In the event, he, those who live in Paris and the military bands that play patriotic music throughout Bastille Day may regret the traffic jams the coincidence will certainly create.

But Mitterrand's gesture is well calculated. There are aspects of the French Revolution that may be overlooked despite the welter of retrospection in which historians have been engaged for the past year and more. Not that the historians' interests are a bore. It remains a matter of fascination that a revolution born of enlightenment (almost literally of the Enlightenment, as encapsulated in Diderot's great *Encyclopédie*) or even of naive idealism (Rousseau), should so quickly have been succeeded by the Terror (in which Lavoisier among others was executed, in 1794) and that the man appointed to carry the revolution to the rest of Europe (Napoleon) should have founded an empire instead. What the historians have not been saying is also interesting: little has recently been heard of the once-fashionable theme that the apotheosis of the ostensible failure of the French Revolution is to be found in Lenin's later exploits, for they seem also to have been a disappointment.

What those who are not French may easily forget is that the revolution sprang not just from social discontent, but from a tradition of rationality going back at least to Descartes, Newton's older contemporary. Seventeenth- and eighteenth-century France was also lucky that two of its strongest social institutions, the Army and the Church, provided means by which able people could practise science, the former by the military colleges that became the *grandes écoles*. The revolution's leading scientist, Laplace, brilliantly united the cartesian and newtonian traditions, but also had the grace to write a popular book on the subject with the breathtaking title *Exposition du Système du Monde*. Lavoisier may have literally lost his head, but his contemporaries and successors, Berthelot for example, kept the flag of rationality flying. Lamarck, now generally scorned, had the wit to spell out that natural evolution is a real process, but the children of the revolution included Pasteur (born in 1822), without whom our own century would have begun impoverished. These traditions would hardly have survived the revolution had not they been part of it, but President Mitterrand will be well within his rights if, next weekend, he boasts about it a little to his guests. □

Scientific misconduct still an unknown

- Audit urged at congressional hearing
- Legal position of journals questioned

Washington

WOULD it not be better for the US scientific community to come up with a convincing measure of the extent of scientific misconduct before Congress begins legislation to create stricter controls? That was the question posed by Drummond Rennie, deputy editor of the *Journal of the American Medical Association* (JAMA), at a US congressional subcommittee hearing on the integrity of scientific research last week.

The subcommittee, of the House of Representatives Science, Space and Technology Committee, stimulated congressional interest in scientific misconduct when it held the first hearing on the subject in 1981. Eight years later, congressmen are still asking the same two basic questions: How common is misconduct? Does more need to be done to combat it?

Unlike many scientists, Rennie acknowledges that congressmen have a right to be "surprised and upset" when the scientific community can produce no clear answer to the first question. Rennie asked the subcommittee to encourage an audit of scientific papers to try to find out if there really is a problem. He sees an audit as the simplest way to "resolve the stand-off between those who allege that cheating is common and wish to impose a federal bureaucracy to police it, and those who say it is non-existent".

The idea of an audit is not new, having been first publicly proposed in 1987 by Walter Stewart and Ned Feder (*Nature* 325, 213; 1987). But the idea has never won much backing.

Last month, Benjamin Lewin, the editor of the journal *Cell*, argued that the cost of uncovering fraud by an audit might be greater than the price paid for dealing with frauds as they come to light (*Cell* 57, 699; 1989). But Lewin's argument presupposes that misconduct is so exceedingly uncommon that a gigantic effort is needed to uncover it. Rennie is not after the one case of fraud in 10,000. He points out that routine audits for the Food and Drug Administration (see *JAMA* 5 May 1989) found invalidating defects in 7-12 per cent of cases, although only a small proportion of these may be due to fraud.

Rennie proposes a pilot audit of 300 papers to see if the prevalence of defects is at the "totally unacceptable level" of one in ten, or closer to one in a thousand which he says the scientific community could live with. Clinical journals are the natural choice because that is where problems

concentrate: while doctors of medicine receive just 28 per cent of National Institute of Health grants, surveys show that they are responsible for 65 per cent of cases of reported misconduct.

Auditors, who could be retired scientists, would look for major errors, such as "whether the patients ever existed and whether the information given in the paper corresponds in any way to the data gathered". They would not, Rennie says, try to "adjudicate complex and arguable questions of science".

To the second basic question, of whether more needs to be done to combat misconduct, congressmen at the hearing received only one specific request, that for better legal protection for scientific journals. The subcommittee was looking at the roles of journals for the first time, with the editors of *Nature*, *Science* and the *Journal of the American Medical Association* providing testimony.

Journals were seen as an important layer of defence, detecting error (more easily than fraud) before it entered the scientific record, providing a forum for disagreement (journals without correspondence columns were urged to provide them), and ensuring that notice be given of research found fraudulent.

But in that latter role, journals fear legal action and surveys have shown that it is very difficult to get them to correct the record even though they are protected by the truth of the factual statements made. Barbara Mishkin, an attorney and former deputy director of a presidential commission on medical ethics, asked the subcommittee for "legislation that would afford immunity for good faith reporting of scientific misconduct by academic institutions and scientific journals". Mishkin foresaw that immunity would extend to reports of disciplinary and peer review committees and to the printing of retractions "requested by academic or government officials", whether or not all authors of a contested paper agree.

Of the journal editors present, Rennie supported immunity for those acting in good faith, but neither Daniel Koshland, editor of *Science*, nor John Maddox, editor of *Nature*, agreed. Both told the subcommittee that new legislation was unnecessary, Koshland pointing out that procedures for dealing with misconduct are being taken much more seriously than in the past and these should be "given a chance before new legislation".

Alun Anderson

New 'integrity' offices appear in Washington

Washington

The new office set up within the National Institutes of Health (NIH) to deal with scientific misconduct already has 77 cases on its books. But representatives of the Office of Scientific Integrity (OSI) are anxious to stress that they do not have an avalanche of fraud on their hands; the figure is simply the total number of all cases transferred to OSI as it took over central responsibility for misconduct from the many agencies of the Public Health Service. According to Martin Blumsack, an OSI staff member, a "case" is a catch-all term that may amount to nothing more than a single letter of complaint. Many of the cases will never amount to anything significant.

Both OSI and the new Office of Scientific Integrity Review (OSIR) made their first public appearances at last week's congressional hearing on the integrity of science (see above). In keeping with its role in overseeing misconduct operations, developing policy and reviewing investigations when they go wrong, OSIR keeps at a slightly greater distance from scientists by living in the office of the Assistant Secretary for Health, rather than at the National Institutes of Health.

Neither of the two new offices is yet fully staffed and both have only acting directors. But although they are both just getting started, a long life is by no means guaranteed. New legislation that may try to take more authority away from NIH is expected soon from the House of Representatives Energy and Commerce Committee.

All misconduct investigations face the fundamental problem of balancing protection of the reputation of those accused (perhaps unjustly) with the need to give a fair hearing to the complaints of a "whistle-blower", whose own career may also be at stake from the fact of having made an accusation. OSI says it will adopt a policy of non-disclosure, neither confirming nor denying that any particular individual is under investigation, preliminary or otherwise.

While this certainly protects the accused (no one would like lists of allegations to be made public), experience with OSI's predecessor shows that whistle-blowers can have a hard time finding out what has happened to their cases, what evidence was or was not examined and why a particular decision was made. Blumsack says that OSI is aware of the risks of an investigation ending in cries of "white-wash" and will try to be flexible.

Alun Anderson

CNRS plans 'modernization'

Paris

THE Centre National de la Recherche Scientifique (CNRS), the principal state organization for basic research, last week published its plans for 'modernization', in line with a government directive in October 1988. The theme, as presented by director-general Francois Kourilsky, is 'better management'.

Although couched in encouraging language, the three-year 'strategic plan' seems destined to create casualties. But researchers will have to wait until February 1990 before the plans are finalized.

CNRS researchers will find some solace in the proposals. There will be no attempt to transform the CNRS into a research council "funding universities in crisis" along "Anglo-Saxon" lines. "This hypothesis has been abandoned", says Kourilsky. "We fully adopt the French model of the CNRS as a research organization." Kourilsky was obliquely referring to the previous government's plans to dismantle the CNRS in order to end the schism between universities, *grandes écoles* (academies) and research laboratories.

The major buzz-words of the new plan are: "decompartmentalization", "clear scientific strategies", "solidarity around major interdisciplinary projects", "mobilization of manpower", "competitiveness" and "partnership". A new programme directorate will be created to ensure that the three-year plan is respected, while boundaries between disciplines will be redefined. "Vast" interdisciplinary projects are being formulated, such as research on the environment, new materials, macromolecules and communications science.

The manner in which the CNRS and its laboratories are run is Kourilsky's most immediate preoccupation. Several 'working groups' have been set up to redefine both central and laboratory management along with a permanent system of internal audits. There is to be a "significant adaptation" of CNRS Paris headquarters, while the new plans include more conspicuous regional administrative delegations. A new secretary-general, Jean-Marie Bertrand, has been appointed to coordinate these administrative initiatives. A document sent to researchers, entitled "The development of human resources", suggests that laboratories should be seen as "small or medium enterprises" and their directors given management training. Priority is given to a re-evaluation of the post of researcher.

At present, different proposals are being considered, including the introduction of fixed-term contracts and permanent facilities for specialized training. But a commitment to "favour the recruitment of young researchers" could run into

difficulties. The starting salary is set at about FF102,000 (\$16,000) per year, while the average age of a recent postdoc is 27 years. Salaries offered by industry are almost twice as high.

The sting in Kourilsky's message is hidden behind the heading: "assuring the competitiveness of laboratories". Laboratory running costs have not kept pace with the need for expensive equipment and computers, he says. As a result, some laboratories are "on the verge of losing the means to carry out competitive research on an international level". But, since "the creation of new laboratories will become strictly dependent on budgetary means", criteria of excellence will be "more rigorous" as from this year.

Research units whose "activity has declined" will not see their grants renewed. This, says Kourilsky, is "normal CNRS procedure". Between 1986 and 1988, CNRS withdrew from 120 research units. Current options mean that "this tendency will unavoidably be increased". As a result, some researchers whose grants have been cut will continue to receive only their salaries for a maximum of two years pending their integration within more efficient groups. This will be of no comfort to researchers in life sciences who had hoped they could appeal against cuts

being made by their departmental director (see *Nature* 339, 326; 1 June 1989). Of prime concern to researchers, but absent from Kourilsky's presentation, is the reassurance that cuts will be based upon the advice of peer committees.

Faced with the need for 'creative accounting', CNRS will impose a moratorium on new projects involving very large instruments until 1991. CNRS at present spends FF560 million of its annual FF9,700-million budget on very large instruments, such as telescopes, at the expense of laboratory running costs. "We have seen the cancellation of approved research in order to protect international projects", said Kourilsky.

Finally, CNRS will be encouraged to reinforce partnerships with universities, with industry and with other European research institutes. The potential for greater complementarity between CNRS research opportunities and university teaching will be exploited through common researcher training programmes and joint laboratories.

At the same time, a new agreement has been signed with ANVAR, the national agency for technology transfer. To facilitate European collaboration, all the French state research organizations will be represented in Brussels through a new agency, CORA (club of assimilated research organizations).

Peter Coles

HIGH-ENERGY PHYSICS

SSC is brought another step nearer reality

Washington

A LONG campaign by US high-energy physicists took what may be its penultimate step last week, with the decision by the House of Representatives to approve \$110 million to begin building the Superconducting Super Collider (SSC). Another \$90 million is authorized for continued research and development on the 16-mile-across, 20-TeV proton accelerator. The sum is \$50 million less than the Department of Energy (DoE) had requested, but the budget item still needs Senate approval, and DoE officials will push to get the whole request included in the final US budget.

Money for SSC construction has been put into the president's budget for the past three years, but on the previous two attempts had not survived passage through Congress. A factor in this year's success was apparently a letter written by Energy Secretary James Watkins to Representative Tom Bevill (Democrat, Alabama), chairman of the Appropriations subcommittee on energy and water development, assuring him that a contribution of \$500 to \$1,000 million, in cash or in kind, towards the SSC would be forthcoming from Japan. DoE officials have expressed confidence all

along that foreign and non-federal contributions would decrease the burden on the US taxpayer, but so far the only formal promise is for \$50 million in kind from India.

While most foreign governments balked at promising support before a decision to build the SSC was made, many congressmen wanted to see firm evidence of foreign interest before they would vote for construction money. The letter from Watkins to Bevill, based on an informal feeling for the possible extent of Japanese help, may have helped to break the stalemate, but may also jeopardize future negotiations not only with the Japanese, who have made no official commitment to cost-sharing, but also with other potential contributors, who could decide their help is no longer needed.

DoE is working on plans to set about securing foreign assistance, and clarifying what participants could expect in return, but Japanese officials are thought to be nervous at the prospect of being invited to contribute the amount suggested. Despite much talk, at least in the United States, Japan has given no outward sign of interest in helping with SSC, but would be uncomfortable at having to say no. David Lindley

SEMICONDUCTORS

Memories provoke new plans

San Francisco

AMBITIOUS plans to create a unique semiconductor company called US Memories Inc. from a consortium of US computer and chip makers are stirring a debate over efforts to counter Japanese inroads into high technology that could dictate the direction of future antitrust laws.

The new venture, announced last week, would employ capital from existing companies with the aim of re-establishing the United States' role as a leading producer of dynamic random access memory chips (DRAMs). These chips are regarded as drivers of many other technologies because of the critical role they play in a variety of electronic products. But while advocates hail the plan as a way to recover from 'unfair' Japanese trade practices, opponents denounce it as both a violation of antitrust law and just plain stupid.

The controversy is shaping up as a heavyweight fight: IBM, Hewlett-Packard, Intel, Digital Equipment Corporation, National Semiconductor, LSI Logic and Advanced Micro Devices have already contributed \$50,000 each. They and other backers are talking of putting up at least half the estimated \$1,000 million necessary to compete with Japanese giants such as Fujitsu, Toshiba and NEC.

Initially, US Memories would manufacture 4-megabit DRAMs based on technology licensed from IBM. To blunt the Japanese edge, backers would promise to buy at least half the consortium's output. DRAMs were pioneered by the US company Intel in the early 1970s. But whereas 11 US companies produced DRAMs in 1980, only two do so now.

The new venture is reminiscent of SEMATECH, a Texas-based consortium set up to conduct semiconductor research and development. SEMATECH was itself a reaction to Japanese successes in getting competing companies to conduct joint research and development at the stage before production begins. Under existing US law, such ventures cannot manufacture products — but US Memories president Sanford Kane, who helped form SEMATECH, hopes to find a way around that obstacle.

Some electronics companies are already crying foul. "This is another deal like SEMATECH where we wrap ourselves in the American flag, scream Japanese and go to do something which isn't right to do", proclaims T.J. Rodgers, president and chief executive officer of Cypress Semiconductor Corporation in California.

He predicts that US Memories will expand into other semiconductor arenas — such as the static RAMs that Cypress produces — and has threatened to sue the new venture for violating antitrust laws.

Indeed, removing such a legal threat is

critical to US Memories. "In order for them to have security in assessing the future, antitrust rules ought to be relaxed", says Thomas Jorde, a law professor and antitrust specialist at the University of California, Berkeley. His work helped to form the basis for the National Cooperative Innovation and Commercialization Act of 1989, introduced by congressmen Tom Campbell (Republican, California) and Rick Boucher (Democrat, Virginia). The bill would allow the government to protect some joint ventures that violate antitrust regulations.

Private parties can at present collect treble damages by winning an antitrust lawsuit. The new bill, now with a House of Representatives subcommittee, would still permit a successful suit to stop the practice — but no damages would be awarded. Jorde says that situation would be similar to provisions in both Europe and Japan.

Campbell stresses that his bill applies only in special cases. He says: "It only guarantees the ability to do a joint venture if the joint venture will not harm competition but improve competition." But Campbell insists the spectre of treble damages must be erased. "It is the liability for damages that chills a lot of American firms' interest in collaborative effort." He argues that Rodgers' actions prove the point. "His ability to sue under existing law is precisely what I'm attempting to cure. The threat of a lawsuit from Cypress Semiconductor may be enough to keep US Memories from happening."

Yet another view comes from George Gilder, an author specializing in the economics of technology. He maintains that antitrust is not the issue. "What it is, is stupid", he says. "And that doesn't have anything to do with antitrust." The US companies already producing DRAMs have excellent products, Gilder asserts, and another venture is just not needed. He notes, however, that some initial backers once produced DRAMs and might use the new company to re-enter the market without high start-up costs. Computer makers also might support US Memories because the added competition would lower chip prices. "Those are interesting interests, but they have nothing to do with the future of American technology", says Gilder.

In the meantime, US Memories continues gearing up for operation and expects a government go-ahead on antitrust matters. But Washington's blessing does not remove the threat of a private suit, and many parties involved in the debate are due in Washington later this month to testify on the Campbell bill. Its fate might decide the fate of US Memories.

Robert Buderli

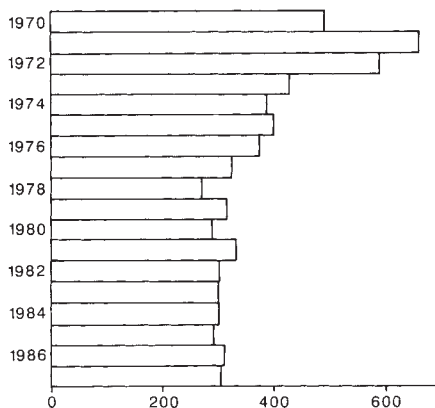
US POSTDOCS

NSF fellows prefer to work at home

Washington

THE number of US postdoctoral fellows supported by the National Science Foundation (NSF) who plan to spend time working abroad has dropped by half since the early 1970s. Recent NSF data show that only roughly 300 postdocs — or just over 2 per cent of all US science and engineering PhD recipients — plan to work outside the United States.

The decline in young scientists' interest in doing research overseas was charted last week at a meeting of the National Science Board, NSF's policy-making body. Richard Ries, who oversees NSF



international affairs, said a fall in the number of US researchers with experience abroad could be damaging to international scientific cooperation, just as international projects are becoming more important. Ries cited the pressure to stay in the academic tenure track and the difficulty in finding US research positions from abroad as reasons given by many postdocs for not seeking jobs in other countries.

Carol Ezzell

ANIMAL WELFARE

Lyons monkeys poorly

Paris

THE 28 stolen monkeys belonging to Unit 96 of the institutes of health and medical research (INSERM) at Lyons, discovered in a private house two weeks ago, have now been identified by the researchers. A spokesman for the research group says that, contrary to what they had been led to believe by police (see *Nature* 339, 648; 29 June 1989), the animals are in a "very poor condition". They are being kept in a zoo and are said to be fighting each other. The tattoo marks that identify each animal have been removed by cutting out the skin; the wounds have not healed and some females may have lost the capacity to suckle. As one team is interested in the developing brain, the animals must be able to reproduce. They will eventually be returned to the laboratory. Peter Coles

US storage site delayed

Washington

THE United States' ability to produce nuclear weapons ran into new difficulties last week when Secretary of Energy James D. Watkins conceded that a new nuclear-waste storage site being dug underneath the New Mexico desert will not be ready on schedule. If the facility is not opened, the United States will have no place to store defence-related nuclear waste after the summer, forcing the Bush Administration to find alternative storage sites or to suspend nuclear-weapons production.

Work on the \$700-million Waste Isolation Pilot Project (WIPP) — a 2,500-foot deep network of caves excavated in underground salt slabs near Carlsbad, New Mexico — has been put off many times since its inception in the early 1970s. The series of caves was planned to hold transuranic wastes (intermediate-level radioactive wastes containing elements with atomic numbers higher than uranium) beginning in late 1988 or 1989. But as construction progressed, the walls of the tunnels began to crack and seep brine, causing the project to be delayed until its long-term safety could be worked out.

In the meantime, the Department of

Energy (DoE)'s Rocky Flats reactor outside Denver, Colorado has been producing 200 cubic yards of transuranic waste per month. Waste from Rocky Flats was supposed to go to WIPP as part of its five-year test period. Idaho has been accepting two boxcars of waste from Rocky Flats each month as an interim measure, because the Rocky Flats plant can store only 1,600 cubic yards.

The Rocky Flats waste has become a contentious issue in the states of Colorado, Idaho and New Mexico as the opening date of the WIPP storage pit has been repeatedly postponed. The governors of the three states accompanied Watkins on a site visit to WIPP last week, but made no concessions to ease DoE's waste-disposal problem. Idaho governor Cecil Andrus has said he will take only two more shipments of waste from Rocky Flats, and Colorado governor Roy Romer has pledged to close the Rocky Flats plant when waste levels reach 1,601 cubic yards.

Watkins, an admiral and former chief of naval operations, is now faced with scaling back production at Rocky Flats, negotiating with the Defense Department to place waste temporarily on a military installation or finding an alternative site. But other sites may be hard to come by: Nevada passed legislation last week banning nuclear waste to prevent DoE from shipping waste to Yucca Mountain, near the underground nuclear testing site. Forcing a state to accept nuclear waste would require a majority vote by both houses of Congress.

Watkins is also in a delicate position because the Federal Bureau of Investigation (FBI) has just begun an investigation of the Rocky Flats plant. The FBI alleges that DoE officials knowingly allowed the plant's managing contractor, Rockwell International, to release radioactivity into the environment and illegally store waste. FBI surveillance planes observed radioactive streams coming from an incinerator and a wastewater plant, and found unmarked barrels of waste. Watkins says that until the waste can be "validated" it will not be moved.

Prompted by the disarray in which he has found his department, Watkins also last week announced a plan to "create a new culture of accountability" within the DoE in environmental protection and waste management. The plan includes steps to bolster DoE's oversight of company contractors and facilitate the involvement of states, clean up current facilities and monitor more closely the effects of DoE activities on public health. Watkins also plans to set up an independent panel to review the WIPP storage site and to ask the US National Academy of Sciences to set up a similar panel.

Carol Ezzell

US—Soviet monitoring

Washington

THE US Natural Resources Defense Council (NRDC) and the Soviet Academy of Sciences are collaborating this week in experiments to assess the ability of passive radiation detectors to contribute to verification of limits on nuclear weapons at sea, the major stumbling block in the strategic arms reduction talks which resumed in Geneva two weeks ago.

The collaboration follows earlier joint projects in which NRDC sent monitoring teams to the Soviet Union to try to show that seismic monitoring could provide an accurate check on a test-ban treaty. NRDC is a private, non-profit environmental-protection organization.

There will be a time limit on access to the warheads and limits on how closely the investigators may approach them. But by allowing US researchers access to nuclear-armed warships, the Soviet Union seems to be making a greater show of commitment to solving problems of verification than is the United States. The experiments will be carried out in the Black Sea, where the teams have access to Slava-class cruisers armed with SS-N-22 SUNBURN cruise missiles. The US government is opposed to the experiment, and the US Navy refuses to confirm or deny that nuclear vessels are on board ships.

Both US and Soviet gamma-ray and neutron detectors will be used to detect the radiation from fissile material in the warheads. Similar experiments are thought to have been carried out by both the US and Soviet military on their own systems and on each others, but no data are available.

The detectors are of only limited use because of the difficulty of using them reliably to detect the radiation from fissile material in nuclear warheads at distances of more than a few feet, and because of the ease with which nuclear warheads can be sheltered from detection. But NRDC hopes to carry out similar experiments with 'active' detectors, which require a radioactive source or a pulsed neutron source. This equipment can detect forms of uranium that passive detection would miss.

Until verification measures are agreed, the United States is opposed to limits on deployment of sea-launched cruise missiles (SLCMs). The Soviet negotiators have proposed limitations of 400 nuclear SLCMs and 600 conventional SLCMs.

Christine McGourty

GENOME SEQUENCING

Flatworm's turn next?

THE above would have been a more appropriate title for last week's item on the US National Institutes of Health programme to map and sequence the genome of the nematode roundworm (*Nature* 339, 648; 1989).

□

ABORTION PILL

RU-486 still troubled

Paris

THE French abortifacient pill, RU-486, is at the centre of debate again, but this time for financial rather than moral reasons. Because the pill, which has been available for about a year, can be used only under strict government control (*Nature* 336, 4; 1988), patients should be able to claim reimbursement under the French social security system. The drug's maker, Roussel-Uclaf, saying that it needs to recoup the cost of twelve years of development, wants to charge FF517 (US\$78), but the Ministry of Health has fixed the value of the drug at FF93.5 (about US\$14).

The Health Ministry says that Roussel-Uclaf has only to widen its market to reduce costs, but anti-abortion lobbies in other countries, notably the USA, have made its introduction outside France too sensitive for the manufacturers. Last year Roussel-Uclaf executives received death threats when the drug was first marketed in France. The product was withdrawn but was subsequently reintroduced after government intervention. Despite the new clash with the Health Ministry, the manufacturers have said they will not withdraw the drug. Meanwhile they are looking at less controversial applications for RU-486, especially its potential as a single-dose contraceptive taken at the end of the menstrual cycle.

Peter Coles

TECHNOLOGY TRANSFER

UK and Japan sign agreement on anti-cancer drugs

Tokyo

UNDER a series of technology-transfer agreements, the latest of which was announced on Monday, the Japanese trading giant Sumitomo Corporation has opened a channel of access for Japanese companies to nearly all anti-cancer drug technology under development in the United Kingdom.

The latest agreement between Sumitomo and the commercial arm of the Imperial Cancer Research Fund (ICRF), a charity and Britain's largest cancer research organization, gives the Japanese company the exclusive right to act as agent in Japan for licensing drugs and inventions arising out of ICRF-funded research. Sumitomo is also able to arrange for Japanese companies to carry out development and clinical trials of ICRF-developed drug technology.

Similar agreements were signed last year with Britain's Cancer Research Campaign (CRC), another charity that funds much of Britain's cancer research, and the Medical Research Council (MRC) (See *Nature* 335, 487; 1988).

The agreements with CRC and ICRF give Sumitomo access to about four-fifths of Britain's cancer research, according to Jonathan Gee, marketing director of Imperial Cancer Research Technology (ICRT), the commercial subsidiary of ICRF which signed the new agreement on Monday.

At present ICRF gets only about one per cent of its income from drug technology licenses and royalties. Most of the charity's research funds come from donations, legacies and income from about 300 charity shops dotted around Britain. But in the near future, Gee expects income from intellectual-property rights to increase substantially. As an example, he points to two ICRF-developed drugs close to commercialization in the United States which are expected to make multi-million-dollar profits — ICRF 1987, a drug which reduces the side-effects of the anti-cancer drug adriamycin, and an antibody-delivered immunotoxin for treatment of graft-host rejection that will be marketed by Xoma Corporation.

But although ICRF has links with about 300 companies worldwide, only about 10 are Japanese, hence the agreement with Sumitomo. Takao Hirose of Sumitomo Corporation says that some contracts with Japanese companies and MRC — the first UK organization to sign up with Sumitomo — have already been entered into and "several companies" are examining CRC drug technology. And he is confident that there will be similar interest in ICRF technology.

David Swinbanks

CONFLICT OF INTEREST

NIH guidance is rejected

Washington

THE US National Institutes of Health (NIH) may already be regretting that they last week hosted a two-day meeting on conflict of interest in biomedical research backed with a promise that they would deliver a new set of guidelines within months. Far from welcoming NIH help, the overwhelming message from the several hundred conference participants was that institutions want to be left alone to tackle the issue themselves.

Diane Zuckerman, a staff member of the congressional subcommittee on human resources and intergovernmental relations, described some of the views expressed at the conference as "very discouraging" and said that Congress may introduce legislation, given that the universities appear to be unwilling to tighten up their policies. Congressional interest in the conflicts that can arise when researchers are in a position to profit financially from their research work is already high, with a subcommittee hearing on the subject held just two weeks ago (see *Nature* 339, 568; 22 June 1989).

No consensus on conflict of interest exists in the universities. Few universities allow faculty to hold equity in a company that finances any of their research. Those that do set varying limits on how much equity can be held. State law in New Jersey prevents faculty at Rutgers University from holding more than one per cent in equity in any company with which it has financial links. A delegate from Rutgers complained that this diminishes the entrepreneurial spirit of the faculty.

California state law requires University of California (UC) faculty to declare on their grant applications any stock holdings greater than \$1,000 in companies sponsoring their research. There is no upper limit on holdings, but a university committee reviews such applications for conflict of interest. The same review process is set in motion if a member of faculty holds a management position with a sponsor, or has received from it more than \$250 for consulting work or as a loan, or more than \$50 as a gift. In seven years almost 30,000 proposals have been examined and only seven have been rejected, although many have been modified before approval.

The process is "extremely burdensome" says Belle Cole, director of research and public policy at UC Berkeley, and costs about \$500,000 a year. But a delegate from UC San Francisco argued that "we prefer to do it within than have it done to us from outside".

The meeting tended to support the view that holding equity in a sponsor was unacceptable, not because it necessarily created a conflict of interest which could bias research results but because of the

public perception that it could. This led to questions on whether restrictions should also apply to family of faculty, and whether holding equity in sponsors' competitors should also be prohibited.

Suggested ways of avoiding the appearance of a conflict of interest included giving responsibility for distribution of the funds from a sponsor to a third party, or placing between a university and a start-up company a third agency to act as a 'buffer' as Johns Hopkins University has done to distance itself from the commercial applications of research. The rule at Johns Hopkins is that the university cannot invest in start-up companies. "We think it's a good rule, but we're still searching for the principle that underlies it", said David Blake, associate dean for administration and planning.

It was agreed that consultancies, gifts and loans were more acceptable, but there were wide differences of opinion over what restrictions there should be. Clinical trials on drugs or treatments were considered by some to require more stringent controls.

In the past the integrity of researchers, the research design and the disclosure of funding sources have allayed concerns about conflicts of interest. But now, researchers in a multi-centre study sponsored by the NIH on the treatment of patients after by-pass surgery have agreed to observe strict guidelines. They and their relatives will not be able to hold stock in companies distributing treatments under study until the results are made public. Paid consulting for the companies is banned. The researchers say similar guidelines should also apply to members of data- and safety-monitoring boards who have access to confidential data.

Clinical trials are already regulated by institutional review boards and often by the Food and Drug Administration and it was argued that no more regulations are necessary. But Bernadine Healy, chairman of the steering committee for the trials, said that guidelines were needed and should be developed nationally for different research projects to provide some uniformity over the academic community. This view was not widely shared.

The question now is what NIH and Congress will do. Researchers at the meeting accused Congress of sending confusing messages to the academic community. Politicians are insisting on closer relationships between federally funded researchers and private industry. But at the same time they are demanding assurances that research remains untainted by the desire for a quick profit and that federal funds are not being used to make researchers rich.

Christine McGourty

Manned spaceflight planned

Tokyo

JAPAN's ambition to put men in space is at last beginning to win serious backing. For the first time ever, the Space Activities Commission, the nation's space policy-making body, last week approved a policy plan for the next ten years that calls for study of manned space flight. But of more immediate significance are decisions by the commission to allow the Institute of Space and Astronautical Science (ISAS) to build bigger solid-fuel rockets for interplanetary scientific missions and to let the National Space Development Agency (NASDA) develop an unmanned cargo shuttle.

Confirmation of earlier reports (see *Nature* 339, 245; 25 May 1989) that ISAS is now free from political constraints on the size of its rockets and can aim at scientific missions to the Moon and planets was well received. Minoru Oda, former director general of ISAS and a key mover behind the policy change, is "very pleased" that the "artificial restraint" on rocket size has at last been removed and sees it as a "really big breakthrough".

Officials at NASDA, which is affiliated with the Science and Technology Agency (STA), are also pleased that the new space policy calls for development of an unmanned space shuttle which will be carried into space on top of NASDA's H-2 rocket. The shuttle, called HOPE (H-2 orbiting plane), will be used to transport cargo to the Japanese module on the US space station and is expected to cost about ¥300,000 million, (\$2,000 million). The US space station is central to the ten-year space policy.

After a prolonged delay caused by Japan's Recruit scandal, the Diet recently approved the US-Japan intergovernmental agreement on the station, thereby allowing NASDA to go ahead with construction of Japan's \$2,000-million module for the station. And as the costs of Japan's space activities rise in the 1990s, the commission hope that Japan's private sector will make significant investments in space.

Apart from the station, the new policy calls for increased international collaboration, for example to monitor global environmental problems such as ozone depletion and the greenhouse effect. And Tateo Arimoto of NASDA's international affairs division is confident that, as a result of the new policy, STA will be able to win funds to begin construction of a large polar-orbiting Earth observation satellite in fiscal year 1990.

The Advanced Earth Observing Satellite (ADEOS), tentatively scheduled to be launched by an H-2 rocket in 1994, will carry sensors developed by NASDA, the US National Aeronautics and Space

Administration, the European Space Agency, and possibly several other countries, and will monitor such things as the ozone layer, wind speed at the Earth's surface and reflectivity of the oceans and land (see *Nature* 335, 486; 1988).

But the new space policy does open up the possibility of conflict between ISAS and NASDA. Until now, the roles of the two space agencies have been clearly defined. ISAS carries out small-scale scientific missions while NASDA

RAIN FORESTS

Germans aid Brazil

Munich & Sao Paulo

In keeping with its newly discovered concern about rain-forest destruction, Brazil has accepted DM100 million (about \$50 million) of grants and low-interest loans from West Germany for the protection of its tropical rain forests. The money will be invested by 1990 to build up the Brazilian Institute of the Environment and Natural Renewable Resources (IBAMA in Portuguese) and to help establish and maintain forest preserves. An additional DM50 million will be given or lent for other environmental projects unrelated to forest preservation.

Money will be spent to support "long-term, ecologically defensible development of the rain forests", a goal that fits in with the current Brazilian trend toward conciliating economic development and the environment. Some of the money will also go to protect the Atlantic coastal forests, which have been reduced to 5 per cent of the area they covered when the Portuguese arrived in the year 1500.

Until early this year, Brazil rejected attempts by developed nations to preserve rain forests. Brazilian leaders dismissed European and North American donors as "environmental imperialists" seeking to meddle in its internal affairs.

The money is expected to be spent on existing projects as well as new projects. West Germany is not giving Brazil a blank cheque; rather, a joint commission has to agree on all proposals.

That West Germany was willing to give the aid came as no surprise; trying to save the rain forests has become one of the most popular environmental causes of the day. In 1988, West Germany announced a special programme of DM250 million in aid for tropical rain forests. The well-publicized hearings of a parliamentary committee on "protecting the Earth's atmosphere" created a "fertile political soil" for giving aid, said Rolf Lerche of the Economic Development Ministry.

Steven Dickman &
Ricardo Bonalume Neto

launches large application satellites. But NASDA officials make no secret of the fact that they, like ISAS, would like to begin missions to the Moon and planets using the soon-to-be-completed H-2 rocket.

Oda says that the H-2 rocket may in future be used for "large-scale" interplanetary or lunar missions. But he hopes that over the next 10-15 years, Japanese scientists will be clever enough to use the "modest" launch capabilities of ISAS rockets. Alternatively, for large-scale missions, total international collaboration may be the best way to go, he says.

David Swinbanks

INDIAN EDUCATION

Gandhi warned

New Delhi

SCIENCE and technology education in India is in a state of crisis, according to Prime Minister Rajiv Gandhi's science advisory council. To improve the quality of education, the council has proposed a \$1,000 million action programme for Gandhi to implement during the eighth five-year plan (1990-95).

There are shortages of manpower in most areas of advanced science and technology, especially in the physical sciences and mathematics, says the council, and there are no institutions in the country that provide even moderately acceptable science education at the undergraduate level. In even the best kinds of institutions, such as the Indian Institutes of Technology and the Indian Institute of Science at Bangalore, facilities are totally inadequate to carry out teaching and research comparable with that in advanced countries, says the council.

In most engineering colleges, the infrastructure is "very poor" and the state of the polytechnics is "deplorable". Most of the money requested would be spent on improving these. The council also recommends the establishment of at least two centrally sponsored but autonomous "model" colleges in each state for undergraduate science students.

After graduating from these colleges, students would take a five-year postgraduate course in selected universities, working closely with the country's leading research institutes. India's national laboratories (of which there are more than 100) could provide postgraduate courses in engineering and technology. The council's chairman, Professor C. N. R. Rao, says this is the only way the country can train the necessary manpower.

The council also suggests the creation of more inter-university centres of excellence, similar to those recently set up for the nuclear sciences and astrophysics. And it proposes the creation of four regional libraries of science and technology.

K. S. Jayaraman

SCIENTIFIC SOCIETIES

Working around the big academy in the Soviet Union

London

SEVERAL new scientific organizations have emerged in the Soviet Union in the past few weeks, at least some of them from discontent with the Academy of Sciences. In May, for example, a group of physicists established the Moscow Physical Society, which they consider to be the spiritual heir of the Russian Physical Society, dissolved by Stalin in 1931.

By organizing conferences and publishing two journals, one specialist, one more general, the society hopes not only to facilitate publication of papers by physicists generally, but also to provide a wider forum for the discussion of matters of public interest. The establishment of the society, the founders note, has ended the anomaly by which the Soviet Union shared with Albania the doubtful distinction of being the only "developed" country without a physical society.

Another anomaly was formally remedied last month with the establishment of the "Russian People's Academy of Sciences". Since the late 1920s, academies on that pattern have been regarded as one of the hallmarks of the sovereignty of the union republics which constitute the Soviet Union.

Yet the largest republic of all, the Russian SFSR, has never had its own academy, although, by an administrative quirk, the Siberian Branch of the All-Union Academy of Sciences is financed from the Russian and not the All-Union budget. Proposals for a separate Russian Academy have been in the air for the past two years, partly as a reaction to the growing ethnic consciousness of the non-Russian republics and under the influence of patriotic Russian scholars such as Academician Dmitrii Likhachev, one of the leading activists in the campaign against the diversion of the Siberian rivers.

Yet another manifestation of Soviet scientists' desire to organize outside the academy network is the new Scientists' Trade Union, the formation of which was announced in Moscow News last month. But the official academy may not be as hidebound as its critics imply. Its monthly *Vestnik* has, in recent issues, displayed a remarkable degree of *glasnost*. The April issue, in particular, began with a dose of self-criticism, saying that it had failed to be a "tribune for discussion" and promising in future to keep its readers informed on important projects undertaken by the academy and of the progress of science overseas. To match the change of policy, 18 out of 21 members of the editorial board have been sacked. Vera Rich

ARCHAEOLOGY

Remains returned to tribe

Washington

AFTER several years of agonizing, Stanford University decided two weeks ago to return its collection of Native American skeletal remains to the Ohlone tribe of northern California, who want to rebury them in accordance with ancestral traditions. The Stanford decision marks the first time such a wholesale return of remains has been accepted by an academic body. But the university's good intentions are already getting it into trouble; ownership of the bones is being contested by the archaeologist who found them in the 1960s.

Bert Gerow, professor emeritus of anthropology at Stanford, says he and his students found most of the bones at two sites in the San Francisco Bay area, and dates them at from a few hundred to a few thousand years old. During that period, says Gerow, there is evidence from the bones and other gravesite artefacts that Ohlone use of the sites supplanted previous occupancy by unrelated Indian groups. He disputes the Ohlones' right of possession.

The Smithsonian Institution in Washington is the biggest institution to have faced the general problem of ownership of archaeological remains. It has a collection of about 18,000 bones from across the country, and maintains a policy of returning skeletal remains of named individuals to identifiable descendants, but such decisions can be made only for bones disinterred comparatively recently. In 1988, the Smithsonian returned to the Blackfeet of Montana a set of bones which, it was proved, had been disinterred from a known gravesite in 1892 by collectors working for the Army Medical Museum.

But most of the Smithsonian's possessions are prehistoric, dating from a time when many groups of Native Americans, especially those of the midwestern and northern plains states, were peripatetic, if not nomadic. Only with the European conquest, and the enforced confinement of groups of Indians to particular areas, did the modern tribal system evolve. For the most part, an association of ancient remains with modern descendants cannot even in principle be made.

In fact, what little is known about the migration and evolution of the early inhabitants of North America has been discovered largely through archaeological investigation, and this has led in some cases to co-operation between scientists and Native Americans who are trying to trace the history and culture of their people. Some more immediate benefits are also claimed: Native Americans suffer from a high incidence of rheumatoid arthritis and osteoporosis, and research on bones may help explain why. But the same resurgence of interest by Native

Americans in their past, which motivates much of the archaeological research, has also increased sensitivity to the treatment of human remains.

There have been efforts in Congress to provide legal guidelines for the disposition of Indian American remains, but they are moving slowly. Beyond the practical difficulties of deciding who owns what, different tribal groups have their own ideas of what constitutes proper treatment. Whereas the Ohlones seem pleased to take the bones from two historical sites in their entirety, the Blackfeet, who got some remains back from the Smithsonian last year, went to great lengths to be assured that what they received were truly their own; having alien remains in a Blackfoot grave would be as irreligious as having Blackfoot remains in the museum.

The Stanford case adds a new twist to battles over the ownership of bones. Gerow says that the Stanford bones have no direct connection with the modern Ohlone people, and belong to him.

Stanford University, on the other hand, has decided that, in this case at least, scientific interest is outweighed by the sentimental if not directly demonstrable link between the Ohlones and their forefathers. But the settlement between the university and the Ohlone tribe, hailed by both sides as an amicable resolution of a delicate dispute, now seems destined to become a squabble in a legal no-man's-land where the university's regulations provide no guidance. **David Lindley**

ERYTHROPOEITIN

Government to pay

Washington

DESPITE initial fears that Amgen's newly approved erythropoietin (EPO), see *Nature* 339, 493; 1989) would be too expensive for government reimbursement schemes, the US Health Care Financing Administration has announced that it will pay for the drug for kidney-dialysis patients. The agency will pay \$40 for each administration, on top of the dialysis costs that it pays for all dialysis patients in the United States.

Amgen's partner in the development of EPO, Johnson & Johnson, announced last week that its Ortho Pharmaceutical division will give the drug free to AIDS patients, under a special pre-approval programme called a treatment IND. J&J has licensed the non-dialysis EPO market from Amgen, and is seeking US Food and Drug Administration (FDA) approval for using it to treat anaemias of any cause. The treatment IND designation was designed by FDA to allow access to drugs before approval to patients with life-threatening diseases. **Carol Ezzell**

Use of animals in research

SIR—Professor Blakemore's attack (*Nature* 339, 414; 1989) on Clive Hollands for expressing his opinion on the publication of work on monkeys (*Nature* 339, 248; 1989) is hysterical and is also a thinly-disguised attack on the freedom of the individual.

Like Mr Hollands, I am a member of the Animal Procedures Committee (APC) created by the Animals (Scientific Procedures) Act 1986. We are more familiar than most with the content and intentions of the act, since we advised the British government at all stages of its preparation and passage through parliament. During this period we campaigned for an advisory committee free from the control of the Secretary of State, so we were delighted when David Mellor MP, then Under-Secretary of State at the Home Office, said in the House of Commons Committee on 13 March 1986 that the APC "will be able to tender advice to the Secretary of State whether or not he asks for it" (*ATLA* 14, 6-13; 1986). The rights of the APC and its members are defined by the law, not by the Home Secretary.

Membership of the APC does not preclude us from speaking out on any issue which concerns us, provided that we do not contravene the sections of the act which relate to the protection of confidential information. Mr Hollands does not mention his membership of the APC in his letter to *Nature*. I hope that Professor Blakemore, and any others who join him in writing to the Home Secretary, will receive the kind of reply I would expect if I asked the Chancellor of Oxford University to comment on the right of members of that important institution to pontificate in the way that we have come to expect of Professor Blakemore.

Like the vast majority of those in the animal welfare movement, Mr Hollands has repeatedly made clear his opinion that violence in the name of animal rights is illegal, unjustifiable and counterproductive. Terrorism in any form angers us no less than it angers Professor Blakemore.

Professor Blakemore says that "medical research is fighting for its survival" and that there are philistine forces which would "stop the progress of medicine". Most of medical research does not involve animal experimentation; much progress is being made, and would continue to be made, without it. This kind of emotional exaggeration damages the case which can be made for properly controlled experimentation on animals.

Professor Blakemore ends his letter with a snide remark about Mr Hollands and "his personal law". The 1986 act does not belong to Mr Hollands, and it certainly does not belong to Professor Blakemore. It belongs to all of us, and the

legislatures of other countries, including France and the United States, would do well to examine it before undertaking much-needed reforms in their own legislation.

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SIR—Professor Blakemore's recent defence of *Nature's* publication of work involving two fetal macaque monkeys and their mothers (*Nature* 337, 265-267; 1989) offers little help in thinking about two serious ethical questions raised by Mr Hollands' criticisms.

The more general of these two questions concerns the publication in Britain of the results of animal research which would not have been allowed under British law. Should British journals publish such work? Blakemore, disappointingly, does not comment on this important issue, preferring instead to concentrate on the question of whether this particular piece of work would have been legal. The second question relates to whether the work was 'justified': were the 'benefits' accruing from the research sufficient to justify the 'cost' (in terms of pains and distress imposed on the animals used)? Mr Hollands clearly believes that the justification for the work was insufficient, or perhaps the work had not been sufficiently justified by the scientists, and so argues that the work would not have been permitted under British law. Professor Blakemore, on the other hand, suggests that the work was justified, but gives little evidence to support his view.

Professor Blakemore states that the work "addresses fundamentally important questions about the mechanisms of development of the cerebral cortex", but we are not informed how the results of this work advance understanding. Similarly, we are told that the work was "performed skilfully and humanely in one of the leading centres for biomedical research". All well and good, but what of the specific effects on the monkeys used in these particular experiments? The results represented are for only two animals — were others used? Only when such specific information is provided can other scientists (who are also part of the general public and like Professor Blakemore have been unwarranted targets of anti-vivisectionist abuse) be persuaded of the work's justification.

I hope that scientists will not in Professor Blakemore's words "mobilize to resist the philistine forces that would stop the progress of medicine", but, where possible, will attempt to meet temperate criticisms

head on, by providing reasoned, detailed and logical answers to reasonable questions. Too often those with some ethical reservation about a piece of animal research are accused of being anti-science. That is simply not true. There are real and difficult practical ethical dilemmas involved in carrying out animal research and most scientists take these seriously. It is for that reason that rational debate on such issues is to be welcomed, and is partly the reason why one university has seen fit to create a department of Biomedical Science and (biomedical) Ethics to promote such discussions.

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■ While *Nature's* largest editorial office is in Britain, the legal basis of its publication is identical in Britain, Japan and the United States — Editor, *Nature*.

Unfair anonymity

SIR—Earlier this year a well known colleague received a rejection notice from a specialist journal whose decision was based on an excessively harsh, anonymous review. The colleague's response was extreme: suicide. Because he suffered from clinical depression, some other event might have eventually precipitated the same response. However, this tragedy highlights a widespread phenomenon. Most of us know individuals whose attitudes towards publication or pursuing a career in science have been marred by a hostile or *ad hominem* review. Most reviewers are fair-minded, but when scientists use an anonymous review as a means of denigrating their rivals, they undermine the spirit of science. Most authors take harsh reviews in their stride, but this means that the publication process favours authors with skin of a particular thickness.

This unwitting selection for scientific rhinoceroses comes at the expense of diversity. I am not advocating lower standards for acceptance of papers — but how many journals explicitly instruct their referees to focus their criticisms on the manuscript rather than its author? How many editors refuse reviews that are primarily personal attacks? How many reviewers look upon the review as a chance to have a positive influence on someone else's research?

Anonymous peer review has its advantages and disadvantages, but should surely be granted only to those willing to accept certain responsibilities.

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The public understanding of science

John R. Durant, Geoffrey A. Evans and Geoffrey P. Thomas

Both in Britain and the United States the public says it is more interested in science than (for example) sport. Public knowledge of science gives less cause for gratification.

WHY should anyone care about the public understanding of science? First, science is arguably the greatest achievement of our culture, and people deserve to know about it; second, science affects everyone's lives, and people need to know about it; third, many public policy decisions involve science, and these can only be genuinely democratic if they arise out of informed public debate; and fourth, science is publicly supported, and such support is (or at least ought to be) based on at least a minimal level of public knowledge. Whether or not the difference between public understanding and public ignorance of science is, as Isaac Asimov has claimed¹, "the difference between respect and admiration on the one side, and hate and fear on the other", common sense suggests that the scientific community would be unwise to presume upon the continued backing of a public that knows little of what scientists do.

So, how much science does the general public understand? The answer that emerges from our research in Britain and that of Professor Jon Miller and his colleagues in the United States is 'not much'. According to the results of two parallel national surveys, carried out in the summer of 1988, levels of British and American public interest in science, technology and medicine are relatively high; but levels of *knowledge* of each area are far lower. Although an impressive 66% of British and 62% of American respondents clearly understood the implications of simple probabilistic statements, only 34% of Britons and 46% of Americans appeared to know that the Earth goes round the Sun once a year, and just 28% of Britons and 25% of Americans knew that antibiotics are ineffective against viruses. Equally striking, in the context of current public and political concern about technology and the environment, is that only 34% of British respondents knew that nuclear power stations are not a source of acid rain, whilst a mere 23% recognized a link between the burning of fossil fuels in coal-fired power stations and the problem of global warming.

Several previous studies have investigated public perceptions of science and technology²⁻⁴, the most systematic of which have been the US National Science Foundation (NSF) *Science Indicators* survey series conducted by Miller over the past decade⁵⁻⁸. In general, previous survey researchers have concentrated on public

attitudes towards technology. For this reason, we have developed a range of new questions which, in combination with existing measures, permit us to explore the public understanding of science and science-based technologies (including medicine). At the same time, we have collaborated with Miller and his colleagues in establishing a limited area of overlap between our own and the

is broadly similar, except that there are consistently slightly higher levels of self-reported interest in science, technology and (especially) medicine.

These results are quite surprising. As a check on their reliability, British respondents were asked to say how likely it was that they would read newspaper stories under a variety of different headlines (Table 1B). Medical stories (headlines c



Science Indicators survey series with a view to international comparison.

Stratified random samples of 2,009 British and 2,041 Americans adults over the age of 18 were interviewed in late June and July 1988. The results make sobering reading for politicians and civil servants with an interest in science policy, for scientists with an interest in their public constituency, and for educationalists with an interest in the dissemination of scientific learning. For brevity and clarity's sake, we shall concentrate here on Britain, making reference where appropriate to comparable data from the US *Science Indicators* study. Full details of the comparison are available elsewhere⁹.

We begin with interest, because this represents a basis for an individual's cultivation of understanding and the formation of attitudes (Table 1A). Respondents were asked to say how interested they were in six different issue areas in the news. Easily the highest level of self-reported interest is that for new medical discoveries; next come the levels for new inventions and new technologies and scientific discoveries; and after these (in descending order) come those for sport, new films and (predictably, perhaps) politics. The pattern in the United States

and I) are clearly in the lead, together with an item on government cuts; and once again, scientific and technological stories (headlines d, f, g, i and k) also have a very strong following.

In general, one would assume that people who are interested in a particular issue will tend to be relatively well informed about it. We asked respondents to say how well informed they were about each of the self-report interest issue areas (Table 1C). As we had expected, self-reported interest and 'informedness' match pretty well for sport, politics and films; but for science, technology and medicine there is a substantial discrepancy: here, only a minority of those who report that they are very interested also claim that they are very well informed. As before, the pattern for the United States is broadly similar, with self-reported levels of informedness running consistently at a slightly higher level than those in Britain. For science, technology and medicine, it appears that many people perceive a gap between themselves and a world of learning about which they would like to know more.

So much, we may say, for the myth that most people couldn't care less about science. Of course, these results must be treated with caution. Simple declarations

of interest are easily made, and it is possible that they represent what respondents thought they ought to say rather than what they actually felt. We were careful to ask the interest and informedness questions before respondents were aware of the purpose of the interview; but even so, people possibly felt pressured to give false answers. That this was not a serious problem is suggested by the fact that the self-report results hang together well and relate sensibly to other measures. Respondents who said they were very interested and very well informed tended to be better educated, and (as we shall see) tended to perform better in objective assessments of scientific understanding.

The independent assessment of understanding is at the heart of our inquiry. We measured scientific understanding on two main dimensions: understanding of the processes of scientific inquiry (henceforth, process); and knowledge of the elementary findings of science (henceforth, knowledge). In combination, we

believe that process and knowledge provide a reasonable index of scientific understanding. Of the two, process is by far the trickier to deal with — the issues are more abstract and less clear-cut, and the task of formulating sensible and serviceable questions is inherently difficult.

We adopted a two-part question that has occupied an important place in the NSF survey series. British respondents were asked whether they had a clear, a general, or little sense of what it means to study something scientifically; and respondents falling into the first two categories were then asked to state in their own words what it means to study something scientifically. The resulting statements were recorded verbatim and then placed into one of four categories (see Table 2, question *a*). The results make rather gloomy reading for anyone who takes seriously the conventional, quasi-popperian model of scientific method. For in their answers less than 14% of respon-

dents made any mention either of theory construction and hypothesis testing, or of the experimental method. These results are broadly consistent with the findings of the NSF survey series.

Before concluding that only a small minority of British adults have any grasp whatever of the roles of theory and experiment in science, we should reflect on the sheer difficulty of this item. Arguably, the question measures linguistic as much as scientific skills; certainly, it ignores the possibility of tacit public understanding of the processes of science.

To get round this problem, we devised a number of questions which required respondents only to recognize and not to state the right answers (Table 2). In general, the results point to considerable tacit public understanding of the processes of scientific inquiry. Although fewer than 14% of respondents mentioned experimentation unprompted, more than 56% opted for the experimental approach when given a choice between alternative methods of investigating a problem (Table 2, question *b*); and although fewer than 4% mentioned theory construction and hypothesis testing unprompted, more than 55% disagreed with the proposition that 'All of today's scientific theories will still be accepted in a hundred years' time' (Table 2, question *f*).

As well as assessing our respondents' grasp of the processes of scientific inquiry, we wanted to find out how scientifically knowledgeable they were. For this purpose the quiz shown in Table 3 was devised. One notable point about this battery of more than 20 factual items is its extremely elementary nature. To be sure, one or two items (such as 'Hot air rises') were included as morale boosters in the expectation that virtually everyone would get them right; but the remainder were selected to fall within the range of significant variation in public understanding of science.

A subset of 15 quiz items from the British study was also included in the US survey. Where 31% of British respondents knew that electrons are smaller than atoms, the comparable figure in the United States was 43%; and where 34% of British respondents were able to state correctly both that the Earth goes round the Sun and that it takes a year to do so, the comparable figure in the United States was 45%. In case these figures should appear overly flattering to the American public, it is worth reporting that where 74% of British respondents knew that some radioactivity occurs naturally, the comparable figure for the United States was 65%; and where 46% of British respondents knew that the earliest human beings did not live at the same time as the dinosaurs, the figure for the United States was 37%. Overall, American scores on the 15 quiz items fielded in both countries

TABLE 1 Measures of interest in and informedness about science and technology

TABLE 1A Self-reported interest

	Very interested	Moderately interested	Not at all interested
<i>a</i> Sport in the news	27.9	42.9	29.2
<i>b</i> Politics	16.2	54.7	29.1
<i>c</i> New medical discoveries	49.0	40.9	10.1
<i>d</i> New films	17.2	38.3	44.5
<i>e</i> New inventions and technologies	39.4	45.0	15.6
<i>f</i> New scientific discoveries	38.2	44.0	17.8

TABLE 1B Self-reported interest in newspaper headlines

	Definitely read	Might read	Definitely not read	Don't know
<i>a</i> Heathrow Robbery and Drugs Deal	54.7	37.8	5.2	2.2
<i>b</i> East Enders Star in Baby Shock	16.6	31.5	44.2	7.6
<i>c</i> New Clue in Hunt for AIDS Cure	63.1	26.5	8.2	2.2
<i>d</i> Robots for Housework Soon	20.1	35.6	33.2	11.2
<i>e</i> FA Cup Winners Surprise	29.6	25.4	37.3	7.7
<i>f</i> Can Pesticides in Rivers be Controlled?	46.7	32.5	12.7	8.2
<i>g</i> Government Backs Massive Investment in Science	38.5	36.7	16.3	8.5
<i>h</i> UN Calls for British Action Against South Africa	38.7	34.0	18.4	9.0
<i>i</i> Astronomers Discover New Galaxy	40.7	29.9	21.9	7.5
<i>j</i> 60's Pop Idol in Comeback	15.8	34.4	37.6	12.2
<i>k</i> "Was Darwin Right?" Say Scientists	33.2	33.4	20.8	12.7
<i>l</i> Heart Disease Our Own Fault Claim Experts	68.5	24.3	5.0	2.2
<i>m</i> New Government Cuts Announced	66.6	24.4	6.3	2.8

TABLE 1C Self-reported informedness

	Very informed	Moderately informed	Not at all informed
<i>a</i> Sport in the news	28.3	41.8	29.8
<i>b</i> Politics	16.8	54.8	28.3
<i>c</i> New medical discoveries	9.9	54.8	35.1
<i>d</i> New films	11.5	36.0	52.4
<i>e</i> New inventions and technologies	9.4	52.9	37.6
<i>f</i> New scientific discoveries	9.0	47.5	43.3

All scores are percentages. The final measure of interest was constructed using six items (Table 1A, questions *e* and *f*; 1B, questions *f*, *g*, *i* and *k*). These were the items that loaded most heavily on the first, unrotated principal component of a principal components analysis of all the interest items. The medicine items (1B, questions *c* and *h*) loaded rather weakly with science; and on a rotated solution, they loaded on a separate dimension with items *a* and *m*. This suggests that interest in medicine is distinct from interest in science.

The six science items were then aggregated to form a scale. Responses in 1A were scored 3 points for 'very interested'; 2 points for 'moderately interested'; and 1 point for 'not at all interested'. Responses in 1B were scored 3 points for 'definitely read'; 2 points for 'might read'/'don't know'; and 1 point for 'definitely not read'. The maximum score on the scale is 18, and the minimum score is six. The mean for the scale is 10.66 and Cronbach's Alpha reliability coefficient is 0.80.

were on average slightly higher than British scores. In the public knowledge of science stakes, it appears that the United States leads Britain by a short head — but we rather doubt whether these figures give much cause for celebration on either side of the Atlantic.

The attempt to measure scientific understanding on two dimensions rests on the assumption that process and knowledge do indeed constitute meaningful components of a single, larger construct: scientific understanding. This assumption is supported by our results, which reveal a close association (by the standards of social survey research) between our aggregate process and knowledge measures ($r = 0.68$). This association justifies the further aggregation of the two scales into a single scale of 27 items (Cronbach's Alpha reliability coefficient = 0.84). It is this larger understanding scale that will be used for further analysis.

What sorts of people tend to know more about science? Using a number of standard socio-demographic measures, and deriving zero-order correlations with scientific understanding, we find that in Britain younger people tend to know more than older people; males tend to know more than females; and middle-class people tend to know more than working-class people. The strongest association of all is between educational level and scientific understanding. Significantly, the same (though generally weaker) pattern of associations emerges between socio-demographic variables and interest in science and technology. Finally, there is a reasonably strong association between interest and understanding ($r = 0.49$).

This is the same broad pattern of associations that has been a consistent feature of the NSF survey series, and in neither case are the results surprising. For example, so far as age is concerned it would be astonishing if the radical improvements in education that have taken place over the past two generations had not left at least some sort of mark on the public understanding of science; and in connection with sex, it is regrettably a commonplace that in our culture women tend to be less interested and involved in science than men. This being said, it is important to establish these associations, not least in order that we can direct education and other forms of public scientific communication more accurately.

How do these results, briefly sketched here, speak to the reasons for caring about public understanding of science with which we began?

On the question of science as a cultural achievement, there is clear cause for concern. Some of the questions about knowledge addressed elementary but fundamental aspects of the scientific world-picture, yet they defeated a majority of

respondents. That most of the public do not know of the achievements of J.J. Thomson, Robert Millikan and Niels Bohr (Table 3, question *i*) may perhaps be explained on the grounds of the relatively esoteric nature of atomic physics; but what excuse shall we give (not for the public, but for ourselves as scientists and educators) to account for the fact that most of the public appears not to have caught up with Nicholas Copernicus and Galileo Galilei (Table 3, questions *u* and *v*)? If modern science is our greatest cultural achievement, then it is one of which most members of our culture are

very largely ignorant.

As regards practical reasons for caring about the public understanding of science, we find a similar picture. Although our survey was not intended primarily to discover how well the British work-force is equipped for life in an economy dependent upon science and technology, the fact (based on ancillary questions in the survey, not shown in the tables) that only between a quarter and a third of our respondents were able to give a minimal account of the difference between computer hardware and software does not augur particularly well. Even more

TABLE 2 Measures of understanding of processes of scientific inquiry

<i>a</i> What does it mean to study something scientifically? Answers including reference to:						
Theory construction						3.2
Experimental method						10.5
Other answers						43.1
Don't know/Not answered						43.2
<i>b</i> Suppose a drug used to treat high blood pressure is suspected of not working well. On this card are three different ways scientists might use to investigate the problem. Which one do you think scientists would be most likely to use?						
(i) Talk to patients to get their opinions						12.5
(ii) Use their knowledge of medicine to decide how good the drug is						26.5
(iii) Give the drug to some patients but not to others. Then compare what happens to each group						56.3
(iv) Don't know						4.4
<i>c</i> When scientists talk about Einstein's theory of relativity, are scientists talking about						
(i) A hunch or idea						11.2
(ii) A well established explanation						33.3
(iii) A proven fact						39.1
(iv) Don't know						16.4
<i>d</i> And when they talk about Darwin's theory of evolution, are scientists talking about						
(i) A hunch or idea						19.7
(ii) A well established explanation						44.6
(iii) A proven fact						21.1
(iv) Don't know						14.7
<i>e</i> Doctors tell a couple that their genetic make-up means that they've got a one in four chance of having a child with an inherited illness. Does this mean that						
	Yes	No	Don't know			
(i) If they have only three children, none will have the illness?	4.9	84.2	10.7			
(ii) If their first child has the illness the next three will not?	9.3	80.3	10.3			
(iii) Each of the couple's children has the same risk of suffering from the illness?	82.1	9.6	8.0			
(iv) If their first three children are healthy, the fourth will have the illness?	8.6	80.3	10.9			
	Agree strongly	Agree slightly	Neither agree, nor disagree	Disagree slightly	Disagree strongly	Don't know
<i>f</i> All of today's scientific theories will still be accepted in a hundred years' time						
	7.1	16.9	13.5	30.4	24.9	7.3
<i>g</i> New technology does not depend on basic scientific research						
	3.8	21.8	16.5	29.3	17.3	11.5

All scores are percentages. The final process scale was constructed using all seven questions. Answers to question *a* were coded into four categories: (i) answers referring to science as a process of theory construction and hypothesis testing; (ii) answers referring to the notion of experimentation, but not mentioning the testing of theories or hypotheses; (iii) other, often rather vague answers referring to science as a process of fact gathering, or mentioning concrete scientific procedures (looking down a microscope, for example); and (iv) respondents who did not qualify for the question (see main text), or who when asked replied 'don't know'.

These categories were used as a four-point scale, with category (i) scoring 3 points and category (iv) scoring 0. In question *b*, respondents choosing options (i) and (ii) or 'don't know' scored 0 and those choosing option (iii) scored 1. In questions *c* and *d*, respondents choosing options (i) and (iii) or 'don't know' scored 0, and those choosing option (ii) scored 1. In question *e*, respondents who stated that option (iii) was correct and that all other options were incorrect scored 1 (the number scoring 1 on this criterion = 66%). In questions *f* and *g*, those disagreeing with the propositions scored 1.

A principal components analysis of the process measures showed them to possess only a single major dimension. The process scale has a maximum score of 9 and a minimum score of 0. The mean score is 3.76, and Cronbach's Alpha reliability coefficient is 0.61.

worrying, in the quiz more than half our respondents asserted that antibiotics kill viruses as well as bacteria, while almost 70% believed that natural vitamins are more efficacious than laboratory-made ones.

We turn next to what might be termed the democratic argument for promoting the public understanding of science. What are the prospects for informed public debate and decision-making when a large

proportion of the public is confused about most of the relevant facts? For example, consider some results from another question not included in the knowledge quiz. We asked whether nuclear power stations cause acid rain. Almost 50% of respondents said they did, and only 34% said they did not.

This result is strongly related to scientific understanding. Only 11% of respondents in the top quartile of scientific

understanding believed that nuclear power stations cause acid rain, whereas the figure for the bottom quartile is 62%.

Though impressively strong, this relationship between knowledge of the environmental effect of nuclear power stations and scientific understanding is not in itself particularly surprising. After all, knowledge about nuclear power is itself a part of scientific understanding. What the result does show, however, is that our general understanding scale is measuring variability that is directly relevant to specific science-related public policy issues.

Finally, there is the question of the relationship between public understanding and public support for science. Our survey contains a large number of measures of attitude. Preliminary analysis of results on these measures indicates that there are important relationships between public understanding and public attitudes, with a tendency for better-informed respondents to have a more positive general attitude towards science and scientists; at the same time, however, it indicates that it is unwise to generalize to any particular conclusions concerning public attitudes towards specific scientific or science-related public policy questions. This being the case, we shall say no more here about Asimov's image of a largely uninformed public caught between hatred and fear of the scientific community. The results we have provided indicate that although the public is largely uninformed, it is also largely interested in science. This is surely a cause for optimism about the scope for improving the public understanding of science. □

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	True	False	Don't know
a The centre of the Earth is very hot	86.3	3.8	8.3
b All insects have eight legs	7.9	83.7	8.3
c The oxygen we breathe comes from plants	59.9	28.3	11.8
d Radioactive milk can be made safe by boiling it	12.9	65.1	22.0
e Lasers work by focusing sound waves	20.1	41.8	38.0
f Sunlight can cause skin cancer	93.5	3.2	3.2
g Hot air rises	96.7	1.2	2.0
h The liver makes urine	25.4	53.1	21.4
i Electrons are smaller than atoms	30.9	23.5	45.3
j The continents are moving slowly about on the surface of the Earth	71.7	8.1	20.1
k The future children of a body builder will inherit the benefits of his training	12.7	76.7	10.7
l Diamonds are made of carbon	58.9	15.5	25.3
m It is the father's gene which decides whether the baby is a boy or a girl	51.2	26.1	22.5
n Antibiotics kill viruses as well as bacteria	54.5	28.6	16.7
o Natural vitamins are better for you than laboratory-made ones	69.6	17.7	12.6
p Common table salt is made of calcium carbonate	36.5	31.1	32.3
q The earliest humans lived at the same time as the dinosaurs	31.6	46.2	22.1
r Which travels faster — light or sound?			
Light travels faster		74.7	
Sound travels faster		18.5	
Don't know		6.6	
s Nuclear power stations produce radioactivity. Is <i>all</i> radioactivity man-made or does some radioactivity occur naturally?			
All man-made		9.2	
Some naturally occurring		74.3	
Don't know		16.4	
t Of the following things (a) Which is the largest? (b) Which is the next largest? (c) Which is the smallest?			
Solar system	Largest 14.1	Next largest 25.2	Smallest 5.4
Galaxy	13.3	35.8	3.6
Earth	6.9	5.8	71.8
Universe	53.5	17.8	1.1
Sun	5.0	5.2	10.2
Don't know	6.9	10.0	7.8
u Does the Earth go round the Sun or the Sun go round the Earth?			
Earth round the Sun		62.8	
Sun round the Earth		30.1	
Don't know		7.1	
v If the Earth goes round the Sun at <i>u</i> , how long does it take?			
One day		16.2	
One month		2.2	
One year		34.1	
Don't know		10.0	
w When scientists use the term DNA, do you think it is to do with the study of			
Stars		1.8	
Rocks		1.8	
Living things		43.2	
Computers		6.9	
Don't know		46.1	

All scores are percentages. Questions a–s, u, v and w were scored 1 point for a correct answer and 0 for an incorrect or a 'don't know'. Item t was scored 1 point for getting all three question parts correct and 0 for all other answers. The knowledge questions were subjected to principal components analysis, which revealed a single major dimension with no interpretable topic-specific or other minor dimensions.

A preliminary knowledge scale was constructed using all 23 questions. Cronbach's Alpha reliability analysis revealed that items b, i and m were not contributing to the reliability of the scale, and these were therefore removed. The resulting 20-item scale (the Oxford Scientific Knowledge Scale) has a Cronbach's Alpha reliability coefficient of 0.81. The scale is normally distributed, with a mean score of 11.44 and a standard deviation of 4.15.

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End of cold fusion in sight

Although the evidence now accumulating does not prove that that original observations of cold fusion were mistaken, there seems no doubt that cold fusion will never be a commercial source of energy.

It seems the time has come to dismiss cold fusion as an illusion of the past four months or so. At the outset, on 23 February, the suggestion that deuterium nuclei can be made to fuse together at ordinary temperatures, if in exceptional circumstances, seemed a brave leap of the imagination. The article on page 29 of this issue by M. Gai *et al.* of Yale University is merely another nail in the coffin of the idea. The Yale group has done its best to replicate the conditions of the original experiments, but has failed to replicate their results. Similar outcomes have been reported from other laboratories. So what has been learned from these hectic months?

First, the negative results now being reported do not imply that the original observations by Stanley Pons and Martin Fleischmann at the University of Utah and by Stephen E. Jones and his colleagues at the Brigham Young University were grossly mistaken. Events may yet show that there are circumstances in which palladium electrodes in electrolytic cells emit pulses of neutrons just as they would if deuterium nuclei were fusing together; early this month, a group at the Los Alamos National Laboratory was wondering what to make of such an observation.

So far, all that is clear is that the original reports do not conceal a recipe for making large (Utah) or even modest (Brigham Young) amounts of power by deuterium fusion. For the many non-scientists who have been excited by the past few weeks, this will be a disappointment. By the same test, the managers of orthodox experiments intended to replicate what happens within the Sun will be relieved.

One striking feature of these events is that, even now, those who have been trying to replicate the original findings are remarkably good-humoured about the time and energy they have spent. Those concerned seem to have found it an inherently interesting exercise. It is not, after all, every day that they find themselves worrying about electrochemistry and nuclear physics at the same time. And it is interesting to have been reminded at first hand of the remarkable capacity of palladium and titanium to dissolve hydrogen, usually known only from books and journals. Moreover, the brief spell in April when it seemed as if cold fusion would permanently divide chemists and physicists has left no trace.

All of us, even bystanders, have also learned a great deal about the difficulty of

counting absolute numbers of neutrons and of γ -rays. The argument between Richard Petrasso and his colleagues at MIT on the one hand and Pons and Fleischmann on the other about the γ -ray measurements have been for many people educative, to say the least. Petrasso and his colleagues (*Nature* **339**, 183; 1989) first complained of inconsistencies in the only published report by Pons and Fleischmann of their observations, were given an incomplete reply (*Nature* **339**, 667; 1989), but on that basis were able to argue (*Nature* **339**, 667; 1989) that the energy channels in the original equipment had probably been miscalibrated and that the energy spectrum is narrower than the resolution of the γ -ray detector would allow.

There is even doubt about the placing of the γ line purportedly resulting from neutron emission which has been variously reported as at 2.2 MeV and at 2.5 MeV. Pons and Fleischmann originally put it at 2.2 MeV, which is what would be expected if the γ -rays come from the conversion of neutrons in water. But now, in their reply to Petrasso, they say they could not have measured such a peak at such an energy, but that it is in any case at 2.5 MeV (which Petrasso disputes on calibration grounds). The best resolution of this dispute would be by independent measurement, but that seems unlikely while attempts to replicate the phenomenon as a whole are unsuccessful. Meanwhile, there will be many who consider the γ -ray signal to have been an artefact.

That point is nevertheless crucial to the unfolding of events after 23 February, when both *The Wall Street Journal* and *The Financial Times* published long accounts of what had been done at Utah and when the University of Utah held a press conference to tell the wider world. (*Nature* owes Pons and Fleischmann an apology for having reported that, on that occasion, they had said that their formal paper had been sent to this journal for publication.)

It is unthinkable that reports of the production of excess heat in such complicated electrochemical cells would, by itself, have been seriously regarded as proof of deuterium fusion. Only the measurement of nuclear particles and products, with the expected energy, could have commanded the interest since shown. Pons and Fleischmann now say that "as we have repeatedly pointed out,

we are well aware of the deficiencies of these spectra", but there are no records of that reservation earlier than the meeting of the US Electrochemical Society at Los Angeles, by which time they had been sent (but may not have read) Petrasso's first draft of his complaint. This is a more serious retreat than they acknowledge.

None of this implies that Pons and Fleischmann have been anything but straightforward. Put yourself in their position if you believe otherwise. If, on 23 February, you had made such an arresting announcement that the whole world was agog, even picking up the telephone would probably engage you in a half-hour conversation with somebody you had never met. Your compelling interest, to gather more data, would be compromised by the inquisitiveness of people asking elementary questions about issues then, in your mind, settled. It is remarkable that Pons and Fleischmann, with all the pressure on them, should have been able to cover so much ground.

So how should they, in the contemporary argot, have played it? It is too easy to say that they should never have given the story of their doings to the financial newspapers, or have allowed a press conference to be held on their behalf. The conventional wisdom, that they should have sent an account of their work to a respectable science journal and then have put themselves in the hands of its referees and editors, is too bland. If people believe they have found a way of changing the world, why should they not tell the world what is in store in their own way?

But there are obvious dangers in such a course, of which the chief is that one may be mistaken. Ordinarily, there is no shame in that: people make mistakes all the time. Ordinarily, there are also colleagues to point to pitfalls in one's path, but potential sceptics may on this occasion have been denied access by the care with which the project was kept secret over five years. It is less easy to accept that one may afterwards be required to accept irksome conditions on how one practises research by an over-confident university; from about 24 February, Pons and Fleischmann might well have decided that they should put their responsibility to the scientific community before that to the organizer of their press conference. Even now, it would be interesting if they made their data generally available, whatever its correct interpretation.

John Maddox

Ciliate through the looking glass

John Galloway

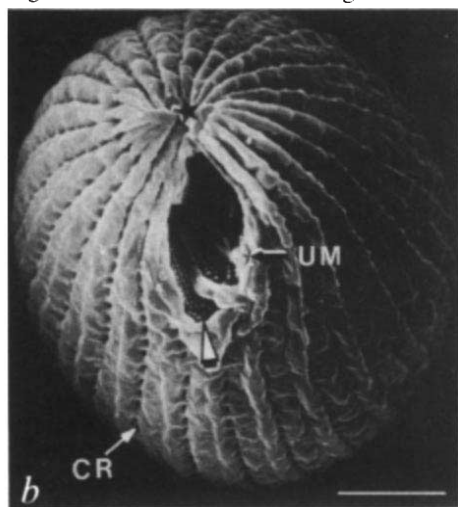
Two papers by E.M. Nelsen and colleagues in a recent issue of *Development*^{1,2} argue that handedness in the ciliate *Tetrahymena* is an example of true cytoplasmic inheritance. Thus are brought together one of the best questions addressed by the science of form, symmetry inversion (the production of a structure's mirror image), and one of the more interesting aspects of inheritance — its disassociation from genetics or at least from nuclear genes, and its replacement by cytaxis — the notion that preformed structure plays a part in the development of the organism. As a sort of bonus, Nelsen and colleagues look at handedness at the level of a single cell rather than the usual extremes of a single molecule or a large assembly of cells.

Ciliates are popular experimental organisms, particularly in the investigation of the control of cellular structure, partly because they possess patterns of cilia on their surfaces which are readily seen and which can be made the subject of grafting experiments — at least in the larger species. Nanney³ points out, however, that the real reasons for their popularity are the examples of cytoplasmic inheritance in *Paramecium* described by Sonneborn and his school. As the seeming conflict between nuclear and cytoplasmic inheritance became resolved to some extent, interest in ciliate genetics waned. But many of the phenomena have not been entirely explained and occasionally new claims of cytoplasmic inheritance are made — as in the papers by Nelsen and colleagues, which make this claim for the inversion of symmetry in *Tetrahymena*.

It is worth comparing symmetry inversion in *Tetrahymena* with that in the gastropods with which it shares some features. In both, a whole organism is being reflected and in both, inheritance is (claimed to be) via the cytoplasm. Gastropod symmetry, however, is known unequivocally to be an example of delayed mendelian inheritance. The hand adopted by an individual depends not on its own genes but on those of its mother. The gene's product, stored in the oocyte cytoplasm, ensures that handedness is preformed through determination of the cleavage pattern (although this is not apparent until the third cleavage). In the ciliate, Nelsen *et al.* claim that an entire system or field of positional information is inherited. In many ways, the interesting problem, of course, is what is the nature of this cytotoxic field? In this respect, it is important to appreciate that the right- and left-handed cells (see figure) are not in fact true mirror images: the cilia are arranged asymmetrically in rows with furrows between them. It is only the arrange-

ment of rows that is reflected; not the arrangement of cilia within rows. This suggests that at least two systems of information must be operating independently, a point made earlier by Grimes⁴ on the basis of his work on the Oxytrichidae, and who suggested the need for three systems.

Independence has unfortunate consequences for the ciliates whose symmetries are reversed from normal. The cell attempts to construct its oral apparatus with a reversed symmetry from constituent parts whose individual symmetry cannot be reversed. These ciliates are unable to feed properly and are placed at a considerable disadvantage compared with



Scanning electron micrographs of right-hand (a) and left-hand (b) *Tetrahymena* cells from an oblique, ventral perspective of the anterior pole (star). CR, ciliary rows; UM, undulating membrane. Scale bars, 5 μ m. (From ref. 1.)

their unreversed colleagues (see figure). Nor are they able to interbreed with unreversed organisms — a property they share (up to a point) with left- and right-handed gastropods of the same species⁵.

It is far from clear exactly what constitutes the field. The idea that it is some sort of explicit spatial template seems unlikely, unless it is very different in *Tetrahymena* than it is in the Oxytrichidae, which undergo a cyst stage during which the whole of the structure de-differentiates and then reforms. Even without knowing the molecular basis for the information system it might be possible to guess some of its features. The simplest idea would be for it to have helical or helicoidal symmetry. In fact Nelsen and colleagues^{1,2} plump for the cylindrical coordinate system of French *et al.*⁶. Provided cellular polarity is specified independently, this would amount to something like the same thing.

Ambidextrousness is a decided feature of the structures of organs, whole animals and plants and now, it seems, of single cells. Ambidextrousness (or lack of it) is of course seen most clearly and most often

among structures that are obviously helical. Among helical macromolecules (proteins and nucleic acids) ambidextrousness does not really feature. The α -helix (and its close relatives), the collagen triple helix, and the A and B DNA double helices are all right-handed. Because amino acids and nucleotides in living things are almost exclusively L-enantiomers, true left-handed mirror image helices do not occur. Nor does any of the helices possess even left-handed counterparts. Z DNA is left-handed, but is in no sense a left-handed version of an otherwise right-handed helix. Bradbury *et al.*⁷ argued that the α -helix of poly- β -benzyl L-aspartate is left-, not right-handed, but this is not a naturally occurring biopolymer. (Many bacterial species are helical; left- and right-handed species are found in the same genus. Whether left- and right-handed

versions of the same species are found is not really known.)

At the other extreme of behaviour lies spiral phyllotaxis — the helical arrangement of leaves on twigs, leaf bases on pineapples and palm trees, scales on pine cones and the logarithmic spiral arrangement of florets in sunflower heads. Left- and right-handedness is assigned entirely at random, shown very convincingly by Davis⁸, and by Rijven⁹ who also discovered that the third leaf from the apex can occupy alternative sites to the right or left of the first leaf. It is here that left-right dissymmetry is introduced, rather as in spiralian development where dissymmetry first manifests itself in the third cleavage.

Somewhere between the strict determinism of molecular structures and the completely stochastic behaviour of phyllotaxis lie *situs solitus* and *situs inversus*. In a small proportion of animals, including humans, normal visceral symmetry is reversed. *Situs inversus*, the abnormal condition, is the result of an autosomal recessive gene but in homozygotes the incidence of *inversus* is about half. Layton¹⁰

proposed that the normal allele specifies normal symmetry and that its absence allows symmetry to be assigned randomly. From ambidextrousness as the result of the loss of genetic control we can move on to the gastropods again, where full genetic control is exercised through an autosomal recessive gene and delayed maternal inheritance.

Temperature is also known to control handedness, as in the Foraminifera. So is oxygen tension, as in developing mouse embryos¹¹. And now Nelsen and colleagues show in the ciliates there is yet another means — some switch in metabolic pathway or whatever — by which symmetry can be reflected. We don't know what this mechanism is, except that it is inherent to the organism, not environmental. On the other hand it doesn't appear to be controlled by genes, if Nelsen and colleagues are to be believed, but nor is it haphazard.

Anyone who has learnt mathematics will know how useful it is to solve the same problem by several different methods

rather than merely use the same method on several different problems. What is true of mathematics is certainly also true of biology. In the long run it ought to be instructive to look at the same phenomenon arising at different anatomical levels across several orders of magnitude — molecule, organelle, cell, organ, organism — and underlain by ostensibly very different sorts of mechanisms. This is especially true when the phenomenon is as elemental as the simple inversion of symmetry. □

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QUANTUM COSMOLOGY

The dichotomy of cosmogeny

Jonathan J. Halliwell

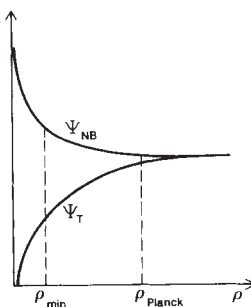
It is only comparatively recently that cosmologists have been sufficiently well-equipped to address in a quantitative fashion the question “how did the Universe begin?”. Although one might have thought that such an esoteric issue lies outside the realm of physics, it has become possible to address it precisely in the field of quantum cosmology, in which quantum mechanics is applied to the whole Universe. There, it becomes the essentially mathematical question “what are the boundary conditions on the wavefunction of the Universe?”. In the search for an answer to this question, much attention has become focused on the issue of deciding between two particular proposals: one due to Hartle and Hawking¹, the other to Vilenkin², Linde³ and others. The consequences of each are so different that it has long been thought that deciding between them should be easy. However, at the auspicious first conference* of the newly created Tufts University Cosmology Institute, L. Grishchuk (Sternberg Astronomy Institute, Moscow) suggested that the issue is not quite so polarized and the truth may lie somewhere in between⁴.

Conventional cosmology, in which the gravitational field is taken to be classical but the matter fields may be treated quantum mechanically, has been very successful in predicting many features of the observed Universe. An important part of the conventional picture is the idea of an inflationary Universe, according to

which it undergoes a period of exponentially rapid expansion during the first 10^{-30} seconds of its existence, before going over to a much more leisurely rate of expansion⁵. In addition to its potential for explaining the flatness of the Universe, the absence of horizons and the origin of large-scale structure, inflation was originally believed to free the present state of the Universe from dependence on initial conditions, essentially because all features the Universe previously possessed are washed out.

However, because this exponential expansion lasts for only a finite period, some dependence on initial conditions still remains. Moreover, the occurrence of inflation itself is very dependent on the initial conditions of the scalar field which drives it — for inflation to occur the

The no-boundary (Ψ_{NB}) and tunnelling (Ψ_T) wavefunctions as a function of initial energy density ρ . The recent results of Grishchuk and Rozhansky indicate that the expressions obtained for these wavefunctions are not actually valid for energy densities in the region $\rho < \rho_{min}$, the region in which the two wavefunctions differ most strongly. (The expressions are also thought not to be valid above the Planck scale ρ_{Planck} but this has been appreciated for some time.)



Universe must start out with a large, approximately constant energy density. Because the curvatures and densities before inflation are so high, quantum gravitational effects are important and gravity cannot be treated classically. Cosmological initial or boundary conditions, therefore, are most appropriately discussed from the framework of quantum cosmology, in which both the matter and gravitational fields are treated quantum mechanically.

The first investigations into the quantum mechanics of closed universes were those of DeWitt⁶, Misner⁷ and Wheeler⁸ in the 1960s. Very much with the issue of cosmological boundary conditions in mind, quantum cosmology was re-vitalized in the early 1980s, primarily by Hartle and Hawking¹ and by Vilenkin². Using elements of an as-yet incomplete quantum theory of gravity, the object is to calculate a wavefunction — the wavefunction of the Universe — which is a function of the geometry of space and of the distribution of matter (for a review, see ref. 9).

In principle, it contains information about the entire Universe and all its material contents, including ourselves. It may be calculated by solving the ‘Wheeler–DeWitt’ equation, the cosmological analogue of the Schrödinger equation in ordinary quantum mechanics.

Much attention has been lavished on simple homogeneous, isotropic models whose only degrees of freedom are a scale factor, representing the size of the Universe, and a scalar field, representing the distribution of matter^{1,2,10}. When the scale factor is large, it is typically the case that the solutions to the Wheeler–DeWitt equation for these models are strongly peaked about a set of classical solutions, some of which have an initial inflationary period. When the scale factor is very small, however, the wavefunction is not peaked about classical behaviour at all, but corresponds to some kind of classically forbidden region.

The picture quantum cosmology offers, therefore, is of the inflationary Universe emerging from a fuzzy quantum mechanical region surrounding the original singularity at zero scale factor, rather like a particle tunnelling out of a barrier. Most importantly, the wavefunction gives a probability distribution on the set of initial conditions for the subsequent classical evolution. The exact form of this distribution depends crucially on which solution to the Wheeler–DeWitt equation one chooses.

Vilenkin², Linde³ and others favour a solution to the Wheeler–DeWitt equation chosen to draw most strongly the analogy with tunnelling in ordinary quantum mechanics. The ‘tunnelling’ wavefunction is large for large initial energy density but small for small initial energy density. This implies that there is a high probability that the Universe will start out with large initial

* Symposium on Quantum Cosmology. Tufts University, Massachusetts, 19–20 May 1989.

energy density — precisely the initial condition necessary for inflation. Hartle and Hawking, on the other hand, favour a solution which is generated using a euclidean (imaginary time) path integral over four geometries (space-times) with no boundary¹. It is calculated approximately by considering the classical solutions to the euclidean field equations. This 'no-boundary' wavefunction is in a sense the opposite of the tunnelling wavefunction, in that it is strongly peaked about arbitrarily small initial energy density, giving inflation a very low probability.

Superficially, it therefore seems that from the point of view of predicting inflation, the tunnelling wavefunction is the correct one. What Grishchuk showed at the meeting, reporting results he has obtained with Rozhansky⁴, is that the issue is not so cut and dried. These authors have carefully re-examined the calculations leading to the above wavefunctions, paying particular attention to caustics — curves along which the neighbouring euclidean trajectories used to calculate the wavefunctions intersect. They find that the approximations used to calculate the no-boundary and tunnelling wavefunctions are not actually valid down to arbitrarily small energy densities, and the Universe fails to become classical when the energy density ρ is less than a certain critical value $\rho_{\min}$. This means, in particular, that the no-boundary wavefunction is not peaked about arbitrarily small initial energy density, but instead is peaked about the critical value $\rho_{\min}$. Whether or not this critical value is large enough to ensure that all classical solutions inflate depends on the details of the model one is considering. But this result lessens considerably the contrast between these two proposals, making it much harder to rule out one in favour of the other.

As Grishchuk emphasized at the Tufts meeting, it would be wrong to get the impression — as one might from the literature — that these two proposals for the wavefunction of the Universe are the only viable candidates. It is perhaps most appropriate at this stage to study broad classes of solutions to the Wheeler-DeWitt equation and ask what sort of properties are generic¹¹. □

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Ancient climate from fossils

Peter D. Moore

CONCERN with atmospheric changes and their possible climatic repercussions is injecting renewed vigour into the study of the climatic changes of the recent past. Particular emphasis is being placed on the dry regions of the world, not only because of their sensitivity to future climatic changes, but also because of their past neglect: unlike the temperate areas, they are not rich in the lake and peat sites that appeal most to palaeoecologists. New sources of evidence are being sought in the isotope record of both plant and animal fossil material, which can provide evidence of past photosynthetic strategies and nitrogen-fixation systems that in turn can be related to climate¹. Even the fossil tooth collagen of ancient grazing animals is now being exploited as a further supplement to the accumulating data, as reported in a recent paper² by Ambrose and DeNiro.

The use of carbon isotopes as a means of recognizing the photosynthetic strategy by which fossil organic matter was fixed is now a well-tried technique. The initial fixation of carbon dioxide by C3 plants, using the photosynthetic enzyme complex Rubisco, discriminates more effectively against the ¹³C isotope than does the primary fixation step of the C4 plant, using PEP carboxylase. The ratio of ¹³C:¹²C in comparison with a standard is given by the ratio $\delta^{13}\text{C}$; for C3 plants this falls at about -26 parts per thousand and for C4 plants at about -12 parts per thousand. Not only can this ratio reveal the photosynthetic strategy involved in the formation of fossil plant material, it can also be used in the analysis of herbivore diets, because the ratio is passed on to the grazer and is laid down in its bone collagen³. This mechanism is particularly useful in the analysis of arid environments and in soils where bone material and its associated collagen are much more likely to survive than other organic matter.

Even snail shells contain sufficient organic residues to provide a basis for carbon isotope analysis. Recent studies in the Negev desert of Israel⁴ demonstrate the potential of such fossil data for revealing palaeoclimate. Snail shells from about 3,000–4,000 years ago have carbon-isotope ratios showing that the northern limit of C4 vegetation was then 20–30 km south of the present limit, indicating that less arid conditions existed then.

Nitrogen-isotope ratios can also be informative. Nitrogen fixed by symbiotic bacteria in legumes has about the same ratio of ¹⁵N:¹⁴N as the ambient air, whereas most non-symbiotic plants have ratios that are higher (richer in ¹⁵N) to the extent of 5 or more parts per thousand. The nitrogen-

isotope ratio of fossil organic matter can therefore be used as an indication of the contribution of nitrogen-fixing organisms to its assembly. As is the case for carbon-isotope ratios, nitrogen-isotope ratios are passed on to consumers, although with a tendency for enhancement by 3–4 parts per thousand (ref. 4). This is, of course, valuable to archaeologists in the study of prehistoric diets^{5,6}. But the findings can also be used in palaeoclimatic reconstruction, as biological nitrogen fixation of this type generally declines in drier soils.

Ambrose and DeNiro² use both techniques in their study of herbivore teeth stratified in sediments from a cave site in the Rift Valley, Kenya. These sediments (5.5 m deep) date from 35,000 years ago to the present day, and the 27 teeth analysed chemically were derived from 10 species of herbivorous mammals (including zebra, duiker, bushbuck and buffalo). Teeth from the upper, younger horizons have isotope ratios closely resembling those of their modern equivalents, demonstrating similar diets and therefore implying that vegetation and climate were similar to those of the present day. But teeth from the lower, earlier Holocene sediments provide collagen with lower ratios of both carbon and nitrogen isotopes than the upper ones.

Most teeth found in sediments dating from 5,000 or more years ago have $\delta^{13}\text{C}$ ratios lower than -20 parts per thousand, and $\delta^{15}\text{N}$ ratios less than +5 parts per thousand. These values are indicative of a largely C3 vegetation in which microbial fixation is responsible for a significant proportion of the nitrogen input to the system. This, in turn, implies a relatively moist climate in comparison to that of more recent times.

Taxonomic analyses of the teeth are in keeping with these findings, for the teeth of Grant's and Thompson's gazelles (open grasslands species) are confined to the upper layers, less than 5,000 years old. Bushbuck and bushpig are now found in grassland and in more forested environments, although they are more frequent in the latter. Their teeth are found to become more frequent with depth of burial, also indicating a moister climate in earlier times.

The results are not themselves particularly surprising; many other lines of

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evidence from East Africa permit similar conclusions, including pollen analyses, geomorphological studies, lake-level changes and the pattern of sedimentation in the eastern Mediterranean Sea. What is of much greater importance is the demonstration that this technique is capable of documenting past animal diets, and hence

COMPUTATION

Making light work of logic

Robert W. Keyes

INTEREST in the dissipation of energy in the logical operations in a computer dates from the early days of transistorized computation, when it was recognized that solid-state devices promised substantial reductions in energy use through miniaturization¹. Thinking about the subject was revolutionized when Charles Bennett explained that dissipation could be reduced to an arbitrarily low level in principle by arranging to do computation in a thermodynamically reversible way². The logic functions commonly used — for example, an AND function with two inputs and one output — are inherently irreversible, however: the output does not contain enough information to allow the physical operations to be reversed and the inputs to be reconstructed. The 'Fredkin gate' avoids this logical irreversibility³. Now G. J. Milburn of the University of Queensland suggests that a Fredkin gate might be implemented with photons, even with single-photon signals⁴.

The Fredkin gate receives three input signals *a*, *b*, *c*, and creates three corresponding outputs, *A*, *B*, *C*. Its logical functioning is shown in the table and can be simply described³. Input *c* passes through the gate unchanged to become output *C*. The value of *c* determines what happens to *a* and *b*: if *c* is zero, all bits are transmitted unchanged; if bit *c* is one, *a* and *b* are interchanged, *a* emerges as *B* and *b* as *A*. It is clear that the inputs can be reconstructed from the outputs.

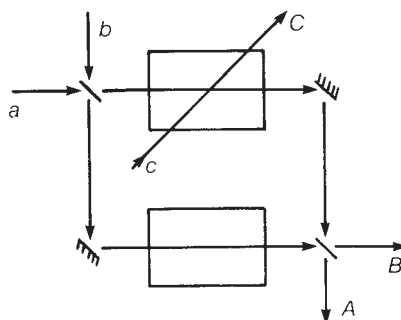
Milburn proposes to operate a Mach-Zehnder interferometer as a Fredkin gate. The Mach-Zehnder interferometer is designed to reveal the difference in optical path length between two beams that traverse different arms of the instrument (see figure). In the optical gate each arm contains a nonlinear optical material, a material whose index of refraction depends on the intensity of the light within it. The signal beams representing *a* and *b* enter the interferometer and are combined so that the intensity in each arm is the same.

The nonlinear element in one of the arms can be irradiated with a beam of light that does not pass through the interferometer and represents input *c*. If there is no external radiation applied as *c*, the beams emerging from the arms interfere to

environments, with such apparent reliability. It offers, therefore, a valuable means of investigating environmental changes in any dry areas where bone or tooth material is available. □

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reproduce the original inputs in separate beams. However, application of light of the correct intensity to this controlled arm changes its index of refraction. The phase of the interferometer beam passing through it is changed, so when the beams interfere at the output the location of the emerging beams is reversed. It is also assumed that the control signal passes through the device unaltered to produce output *C*. If each beam contains only one photon,



Mach-Zehnder interferometer, as modified by Milburn⁴ for reversible logic operations. Input (*a*, *b*) and output (*A*, *B*) signals are split and mixed by half-silvered mirrors; by changing the refractive index of nonlinear optical material, signal *c* alters the path length along one arm of the interferometer.

one has a single-photon Fredkin gate.

Logical reversibility is no guarantee of dissipationless logic; the devices used to build the gate must themselves be free of energy dissipation. In this case, the photons emerging from the single photon gate are presumably identical to those entering, so no energy is lost in the components, and the operation is dissipationless. The use of photons effectively insulates the gate from thermal noise. Photons in the optical region of the spectrum have an energy at least 100 times the thermal energy kT , so that they are unlikely to be produced by a thermal fluctuation.

Milburn is quite frank about the practicality of the proposal. He points out that optical nonlinearities are notoriously weak and that a strong electric field cannot be expected from a one-photon signal. Careful adjustment and very accurate beam-splitting is needed to achieve just the right phase relations. Error-free operation demands that the system be lossless.

One may also wonder what is necessary

Logic table for a Fredkin gate					
INPUT			OUTPUT		
<i>c</i>	<i>a</i>	<i>b</i>	<i>C</i>	<i>A</i>	<i>B</i>
0	0	0	0	0	0
0	1	0	0	1	0
0	0	1	0	0	1
0	1	1	0	1	1
1	0	0	1	0	0
1	1	0	1	0	1
1	0	1	1	1	0
1	1	1	1	1	1

to ensure that all of the photons are in the right place at the right time. Comparison with the so-called single-electron transistor is instructive⁵. In this device, which there is no room to describe here, an electron can sit on a capacitor until it is needed to influence a current. There is no analogue to a capacitor in optics.

What of strong signals containing many photons? More work remains to be done with realistic many-photon pulses. Any fluctuations in the number of photons in the beam would affect the phase shift in the nonlinear optical materials and degrade the operation of the gate. Noise or degradation cannot be tolerated in true dissipationless computation. The point is not to perform a single operation and show that it worked, but to carry through a long series of operations, record the result, and then recover stored energy by reversing the computation². If the sequence is long enough, dissipative end effects associated with recording the result contribute little to the energy cost per operation. So I do not agree with Milburn that no more is required than that "the change in intensity is resolvable above the noise level". In fact, the output of one logic element becomes the input to another. Inputting the noise to the next stage allows the noise to cumulate and grow and produces quick degradation of distinctions between zeros and ones. Currently used dissipative systems control noise with nonlinear response, degrading weak signals (noise) and enhancing strong ones.

In spite of the limitations of the optical Fredkin gate, carefully explained by Milburn, it is fodder for journalists who seek to exaggerate illusions of technological revolutions. According to one report⁶, for example, the development "could have long-term implications for the computer industry" and "might one day enable computers to be built which are enormously more efficient than today's". Milburn himself states that "the model of this paper is offered as a reasonably simple way to explore in more detail possible general quantum limits to computation". □

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Regulation by fish red cells

Andrew Cossins

MUCH attention is being paid to intracellular pH as a means of regulating cell function. Particularly good evidence of this strategy has come from an unexpected source, fish red blood cells. In mammalian red cells, intracellular pH (pH_i) is heavily dependent, among other things, on the operation of a high-capacity Cl^-/HCO_3^- exchange mechanism¹ which, according to all the textbooks, greatly enhances the CO_2 -carrying capacity of the blood. This ability to equilibrate base equivalents means that the pH of the intracellular compartment is always tied to, but is somewhat more acid than, that of the extracellular medium (pH_o), so that plasma acidosis leads to intracellular acidosis. Motais and colleagues, however, report in a recent paper² that red cells of the rainbow trout are able effectively to uncouple the intracellular and extracellular compartments despite the presence of a powerful anion-exchange mechanism. This is achieved not by interfering with the operation of the anion exchanger but by the hormonal activation of a Na^+/H^+ exchange and by a recycling of the CO_2 formed by extracellular dehydration of bicarbonate.

Plasma acidosis poses a particularly difficult respiratory problem for fish. This is because many of their haemoglobin isoforms are uniquely pH-sensitive in their oxygen-binding affinity and capacity, both being greatly decreased with acidification to the extent that saturation is not achieved even at atmospheric pO_2 (the 'Root' effect; ref. 3). This phenomenon is important in the secretion of oxygen both by the swim-bladder and the choroid rete. In addition, fish possess large amounts of white fast twitch muscle, a highly active anaerobic tissue which during sustained, fast swimming leads to the production of lactic acid. The resulting plasma acidosis should lead to an erythrocyte acidosis which, by reducing the oxygen capacity of haemoglobin, puts respiratory function at risk. For many years it was thought that the disruption of oxygen loading at the respiratory surface by acidosis was a major factor limiting responses of fish to stressful situations.

A particularly unusual characteristic of fish red cells has only recently been recognized, namely their extreme sensitivity to adrenergic agonists. Addition of isoproterenol to cells *in vitro* in a saline buffered with HEPES or imidazole leads to the rapid induction of a normally quiescent Na^+/H^+ exchanger in the red cell mem-

brane by means of an adenylyl cyclase-cyclic AMP system^{4,5}. Some idea of the power of this system can be gauged from the fact that intracellular Na^+ increases from about 10 to 50–80 mmol l^{-1} in only 5–10 minutes. This is accompanied by a significant intracellular alkalization and extracellular acidification of 0.1–0.2 pH units within 2–3 minutes despite the presence of intracellular proteinaceous buffers, including haemoglobin. Following stimulation, pH_i and pH_o remain stable for at least an hour.

This adrenergic alkalization of red cells increases both oxygen-binding affinity and capacity^{5,6}; and this should enhance

exchange mechanism, or which potentiates the activity of the Na^+/H^+ exchanger.

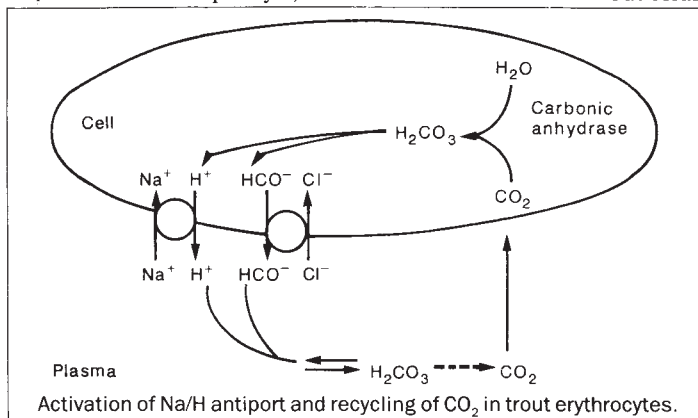
Motais and his colleagues² find that the cause is neither of these. By incubation of blood obtained by catheterization of undisturbed fish (unstimulated cells), they firstly confirm that isoproterenol *in vitro* causes the same large extracellular acidosis and constancy of an elevated pH_i as observed *in vivo*, thus excluding the production of some other hormonally activated system. Second, they show that this is achieved without any change in the partial pressure for CO_2 . Their crucial observation is that addition of carbonic anhydrase to the HCO_3^- -containing plasma virtually abolishes the huge swings in pH_o immediately following adrenergic stimulation, so that the changes in pH_o now resemble those seen in normally buffered but bicarbonate-free salines.

The rapid excretion of H^+ into the bicarbonate-buffered plasma generates H_2CO_3 , but this observation indicates that in normal plasma, which does not contain carbonic dehydrase, the spontaneous dehydration of H_2CO_3 to form CO_2 is sufficiently slow to permit a long-lasting, strongly acidic and non-equilibrium pH_o . Because pCO_2 remains constant, Motais *et al.* suggest that the CO_2 produced by dehydration diffuses into the cell, where it is rapidly converted by erythrocytic

carbonic anhydrase to H^+ and HCO_3^- to fuel further exchanges of the Na^+/H^+ and Cl^-/HCO_3^- antiporters (see figure). The export of H^+ and HCO_3^- replenishes the external concentrations that are reduced by the ongoing dehydration reaction.

Because the extracellular dehydration reaction is rate-limiting, the concentration of extracellular HCO_3^- hardly changes during the acidification phase. Consequently, there is no driving force for the movement of HCO_3^- out of the cell and the anion exchanger is therefore unable to titrate the extracellular acidification. This new hypothesis, combining the activation of a powerful Na^+/H^+ exchanger with rapid CO_2 recycling, explains how Na^+ and Cl^- continue to enter the cell during both phases of adrenergic activation but at the same time preserving pH_i independent of wild swings in pH_o .

This complicated story has at least one important lesson for those brought up in



respiratory performance during stressful episodes. But intracellular pH is still tied to that of the extracellular medium so that the adrenergically induced alkalization of 0.1–0.2 pH units would be swamped by plasma acidosis. Moreover, the export of protons from the cell in itself causes a reduction in extracellular pH which on equilibration of HCO_3^- offsets, to a large extent, the observed increase in intracellular pH. Thus on the basis of *in vitro* analysis it looked as though the functional advantages of the adrenergic system during acidosis were marginal at best.

Exposure of trout to hypoxic water causes the *in vivo* induction of the Na^+/H^+ exchanger⁷, although with some interesting differences to *in vitro* stimulation of cells in buffered salines. Thus, hypoxic induction leads to a far greater extracellular acidosis of 0.6–0.8 pH units within 2 minutes, followed by a slow recovery phase over the succeeding hour. Yet despite this intense extracellular acidosis, the internal pH of the erythrocyte is increased by 0.1–0.2 and is held surprisingly constant during the recovery phase^{7,8}. Thus, the normal relationship between internal and external pH no longer occurs, giving rise to the idea that there was some other plasma-borne factor which either interferes with the normal equilibration of HCO_3^- via the anion-

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the era of molecular biology — that the proper interpretation of cellular function benefits greatly by placing them within a whole-animal context and this applies especially to cellular functions involved in whole-animal physiology. By not enquiring of the role of adrenergic responses *in vivo*, an important and unexpected aspect of cellular pH regulation would not have been discovered. Yet again

HCO₃ has confused researchers, thereby emphasizing Thomas's advice in his recent News and Views article⁹ that "he who works in HCO₃-free media risks studying cellular pathology rather than physiology". □

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THUNDERSTORMS

Electrification of anvil clouds

Anthony Illingworth

In a thunderstorm, the heaviest precipitation and most of the lightning are found in the main convective core. When the vigorous updraught reaches the stable stratosphere it spreads out horizontally forming a characteristic flat-topped anvil-shaped cloud which can extend downwind for tens or hundreds of kilometres. Recent balloon measurements by T. C. Marshall *et al.* (*J. geophys. Res.* **94**, 2171–2181; 1989) and G. J. Byrne *et al.* (*J. geophys. Res.* **94**, 6297–6307; 1989) reveal that these anvil clouds also contain large amounts of charge and extensive regions of high field.

For the main convective core of a thunderstorm, where the radar reflectivity is a maximum, the electrical structure is well established. It consists of a vertical dipole with a positive charge several kilometres above a lower negative charge (Fig. 1). An intracloud lightning flash neutralizes about 20 coulombs (C) of the charge in the dipole; a flash to ground brings some of the lower negative charge down to Earth. It is believed that the active charge separation occurs in the main updraught: small ice crystals are swept upwards by the rising air and are charged positively as a result of rebounding collisions with larger hail pellets. Because of their different terminal velocities the positively charged ice crystals accumulate towards the top of the cloud, while the negatively charged precipitation falls to lower altitudes.

The active core of a thunderstorm is a highly turbulent region with hail and frequent lightning, posing an obvious

threat to aircraft. In contrast, conditions within the anvil cloud are much more placid: small ice crystals are found and turbulence levels are low. It had long been suspected that some crystals, charged by collisions in the main updraught, would end up in the anvil, but the new *in situ* measurements reveal that enormous

50 km from the core. The charge layer was 1–2 km thick so that each square kilometre of cloud contained about 1 C.

All the balloon flights found direct evidence of a screening layer of negative charge at the cloud boundary both above and below the positive charge in the anvil. This screening layer has a similar charge density to the inner positive charge layer and forms when negative ions in the clear air are driven into the cloud by the field, but very rapidly attach themselves to cloud particles and lose their mobility. In one case, the authors could estimate the optical visibility at the cloud edge (450 m) and the screening layer thickness (1,100 m), confirming theoretical predictions that the screening layer at the conductivity gradient of the highly insulating air and the clear air should have a thickness about 2.5 times the optical visibility.

During the past few years, systems for detecting and locating cloud-to-ground lightning have been installed in several countries. Most such flashes bring down

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

FIG. 2 A lightning flash running along the underside of an anvil cloud as recorded by Marshall *et al.* (Courtesy of David Rust, National Severe Storms Laboratory, Oklahoma.)

amounts of positive charge, perhaps several hundred coulombs, are stored in anvil regions, and over large regions the electric fields are over 50 kV m⁻¹.

Two different types of field-measuring apparatus were mounted on the balloons in the new studies. Byrne *et al.* measured the corona current flowing through a conducting-wire probe exposed to the field. Marshall *et al.* devised an ingenious arrangement involving a set of rotating dumb-bells; the charge flowing from one sphere of the dumb bell to the other providing a measure of the field in the cloud. The vertical field profiles observed by the balloons had a similar structure, indicating an extensive horizontal layer of positive charge in the anvil cloud. The inferred charge densities of about 1 C km⁻³ are of similar magnitude to the value in the main convective core, and persisted up to

negative charge to ground, but occasional flashes bringing positive charge from the anvil down to ground are observed. These flashes are amongst the most dangerous, having the highest currents, and extending horizontally for many kilometres. Lightning flashes in anvils are relatively infrequent, and they are especially unexpected, because they tend to occur during the dissipative stage of the storm (Fig. 2). A much higher percentage of positive ground flashes is observed in thunderstorms during the winter but the reason for this is not clear. It may be that in the mobile winter airstreams there is more windshear and the anvil is separated more efficiently from the core of the storm. □

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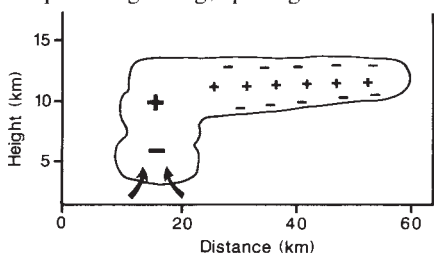


FIG. 1 Charge distribution in a thunderstorm deduced from balloon measurements, showing the electrical dipole forming in the main updraught and positive charge accumulation in the anvil. A negative screening layer forms at the top and bottom of the anvil cloud.

Dinosaur finds from China

Henry Gee

THE intricate history of Mesozoic China is just beginning to yield to the persistent hammers of the Dinosaur Project, a joint Sino-Canadian initiative to explore Jurassic and Cretaceous sediments in Asia and North America. The idea that China in Upper Jurassic and Lower Cretaceous times (175–100 million years ago) was isolated from other parts of the world is gaining credence, thanks to some spectacular fossil finds in the remote Junggar Basin in the Xinjiang region of north-western China over the past three years.

Lower Jurassic faunas from China looked much the same as those from other places, according to Chang Mee-Mann, Director of the Institute of Vertebrate Palaeontology and Palaeoanthropology (IVPP) in Beijing, but Dale Russell of the National Museum of Natural History in Ottawa, just back from Xinjiang, reports that Upper Jurassic stegosaurs and sauropods from China are very strange compared with those from North America.

Another member of the expedition, Don Brinkman of the Tyrrell Museum of Palaeontology in Drumheller, Alberta, notes the presence of an unusual temnospondyl amphibian, indicating that the peculiarities are not confined to dinosaurs. The temnospondyls, large, crocodile-like amphibians, persisted into Upper Jurassic times in China and Australia long after their relatives elsewhere had become extinct. Like Australian marsupials today, the archaic temnospondyls and unusual dinosaurs and fish may be the results of isolation on an island continent (see Andrew Milner's recent News and Views article in *Nature* 338, 117; 1989).

One of the more unusual sauropod dinosaurs is *Mamenchisaurus*, with the longest neck of any animal, living or extinct (see figure). A *Mamenchisaurus* skeleton was one dinosaur in an exhibition of six from the IVPP that drew large crowds at the British Museum (Natural History) last year. The Junggar Basin team were excavating the neck of another specimen, or a close relative, when the political upheaval in Beijing forced the Canadians in the team to leave the country. Team leader Zhao Xijin and his colleagues were left to continue the dig.

Recent events may also jeopardize a return visit to Inner Mongolia in August. A huddle of juvenile ankylosaurs of the genus *Pinacosaurus* were found there last year, and Chang hints that many more exciting finds await discovery.

The period of China's isolation came to an end in Middle to Upper Cretaceous times, when eastern Asia and western North America were united by a Bering Straits land bridge. Even so, there are still many mysteries to unravel. For example, why were advanced ceratopsians (horned

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Sticking its neck out — *Mamenchisaurus* had the longest neck of any living or extinct animal. A neck attributable to this genus, or a close relative, has been discovered recently in northwestern China. (Courtesy of the British Museum (Natural History)).

dinosaurs such as *Triceratops*) so abundant in North America, when only their primitive relatives such as *Protoceratops* and psittacosaur are found in Asia? Small theropods are found in both areas, as are hadrosaurs (duck-billed dinosaurs). The regional hadrosaur faunas are very distinctive nonetheless; where China has *Shantungosaurus*, Canada has *Sauroplophus*. Russell suspects that the answers to some of these questions lie in as yet unknown subtleties of tectonics.

The immense dinosaur diversity in China, first made clear by a British Museum (Natural History) expedition in 1982, is what has impressed researchers most. In Russell's opinion, China was the central heartland that kept a peripheral North America stocked with dinosaurs in the Cretaceous. The sustained joint research effort has forged many strong links between China and Canada which, it is to be hoped, will weather political troubles. Most of us grew up familiar with names such as *Tyrannosaurus* and *Triceratops*. Tomorrow's children will presumably be equally conversant with *Tuojiangosaurus* (a stegosaur) and *Tsintaosaurus* (a hadrosaur). □

Henry Gee is on the editorial staff of *Nature*.

Swinging high

ONE proposed space experiment features a satellite with a long cable, by which instruments can be lowered into regions of aerospace too dense for the satellite itself. Daedalus is taking this idea to its extreme, and is wondering what use could be made of a satellite cable that reached right down to the Earth's surface.

His answer is free transport. A cable simply hanging from an orbiting satellite and tearing across the ground at 8 km s⁻¹, is not an ideal object to hitch a lift on, even if you could catch hold of it. The same cable as an elastic pendulum is much more hopeful. DREADCO's dynamicists are studying the theory of such an elastic cable, dangling from its satellite with a cable car on the end of it. With a suitable combined swinging and stretching motion, the car should at one extreme of its travel lag behind its satellite and stretch downwards on its elastic cable, touching the Earth at rest relative to the surface. The contracting cable would then lift it off again, and speed the car up to swing ahead of its satellite before bringing it down on another point on Earth. Freight or intrepid travellers who entered the car at the first landfall would be carried up in a big cycloidal arc through space, and deposited safely at the second. The energy for their upward journey would be borrowed from the kinetic energy of the satellite and the potential energy of the stretched cable, and repaid back to the system during their descent. In principle, this elegant mode of travel consumes no energy and is truly free.

In practice there are bound to be losses: elastic hysteresis in the cable, and some air drag on the cable car (though it is moving at its slowest while in the atmosphere). A rocket motor on the satellite, fed by fuel pumped up the cable, should maintain the whole system in orbit; small motors on the cable car might also be needed to steer it towards a designated landing site. The cable is the hardest component to design. A uniform cord, even of the best and most elastic fibre material, would break under the take-off loading. But a tapered cable, each section just thick enough to support the sections beneath, should solve the problem.

How to choose the orbit of the new vehicle? A simple circular equatorial orbit would touch few points of importance; an inclined elliptical one would be better. The resulting global itinerary would be rather bizarre. Travellers might have to wait a day or so before the cable car visited a landing site in their neighbourhood; and they might have to hop wildly round the Earth for several orbits before it came down conveniently near their destination. Even so, it should waste much less time than long-haul flying. And the view would be breathtaking.

David Jones

Detection of irradiated food

SIR—In searching for a reliable detection method to identify irradiated food¹, Moriarty *et al.* suggested a test based on the thermoluminescence (TL), emitted on heating irradiated spices from ambient temperature to below combustion². The authors, however, admitted that the errors in their study were still too large to be satisfactory, and other doubts have since arisen³. We now report that it is the inorganic dust in spices that is mostly responsible for TL and that an improved test can be based on the separated dust.

Fine-grained dust was separated from various spices (cumin, green pepper, sage, rosemary, lovage, oregano, dill weed and savory) by centrifugation following wet-sieving in an ultrasonic bath of analytical grade acetone. About 1.5 mg of dust

of the dust samples showed that the TL response is highly reproducible, with coefficients of variation ≤ 5 per cent (90 per cent confidence level). On the basis of isothermal decay experiments at 60, 100 and 150 °C, the half-life of the TL signals was determined to about 5 years at room temperature.

Ultraviolet light was also found to give rise to TL but with a different glow peak temperature of about 175 °C (see figure). This TL may account for the report³ that some non-irradiated samples are identified as irradiated by TL.

In conclusion, TL measurements on

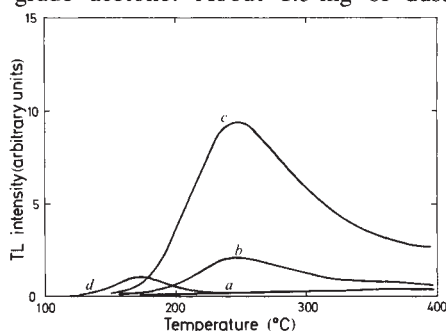
inorganic dust separated from spice samples should allow reliable identification of irradiated spices. The method can probably be extended to other food that contains dust.

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TL glow curves of dust samples collected from irradiated sage. Measurements six days after irradiation at room temperature and subsequent storage at 60 °C in a desiccator. a, unirradiated; b, 1 kGy; c, 10 kGy; d, ultraviolet exposure.

particles is obtained from 3 g of spice. The dust particles were deposited as a 5 μ m-thick layer on aluminium disks (5 mm in diameter, 0.5 mm thick) by evaporating the acetone in a desiccator. After exposing whole spices or separated dust samples to absorbed doses of 1, 10 and 20 kilograys (kGy) at a ⁶⁰Co γ -ray source (Gammacell 220, AECL, Canada) at a dose rate of about 3 kGy h⁻¹, TL analysis (Harshaw 2000) of the dust samples was performed with a linear heating rate of 8 °C s⁻¹, an upper read-out temperature of 400 °C, and with dry nitrogen gas flushing the measuring cell. The aluminium disks fit the dimensions of the heater planellet.

Dust samples were found to emit a significant TL signal only if prepared from irradiated, rather than unirradiated, spices. TL measurements up to 400 °C or more did not cause detectable changes in either the appearance or radiation response of samples, suggesting that the separated dust is inorganic.

The thin layer of the dust samples allowed the structure of the TL glow curves to be resolved. Most had a single maximum between 225 and 250 °C (see figure). Up to about 10 kGy, but not above, a distinct increase in TL output was found. Repeated irradiation and evalua-

SIR—Thermoluminescence (TL) is one of several phenomena being investigated to develop reliable methods for identifying irradiated foods. Repeated reports^{1–5} have claimed that herbs and spices themselves exhibit TL; and this has been proposed as the basis for official testing in West Germany. Our own feasibility work from 1986 onwards demonstrated the strength and reproducibility of TL signals from soil adhering to potatoes irradiated at a typical dose for inhibiting sprouting (0.1 kilogrey). We soon came to the view that these results, taken together with observations of positive signals from freshly irradiated herb and spice samples, could be adequately explained by the presence of mineral grains adhering to sample surfaces⁶. This has been confirmed by our experimental work over the past two years, full details of which are in press^{7,8}. Our purpose here is to summarize the evidence for this view and to show that it has important practical consequences for identifying irradiated foodstuffs.

The TL responses of more than 200 samples of herbs, spices, seasonings and vegetables have been examined before and after gamma irradiation with our 200-TBq ⁶⁰Co facility. TL glow curves recorded at 6 °C s⁻¹ from ambient temperatures to 500 °C show a remarkable consistency of

form. With the exception of seasoning mixtures containing salt (which produces its own glow shape) all samples show similar characteristics to those associated with feldspar and polymineral loess samples in TL dating. Dating studies using similar equipment⁹ have shown that these minerals have high-temperature TL sensitivities, ranging from 10³ to 10⁶ photons s⁻¹ mg⁻¹ Gy⁻¹. Even allowing for saturation effects in the 1–10-kGy dose region, microgram quantities of such materials could easily account for all the signals observed from herbs and spices.

Many samples can be correctly identified as irradiated or unirradiated on the basis of signal strength alone. More than 90 per cent of irradiated samples can be identified using thresholds just greater than 1,000 counts s⁻¹ mg⁻¹. Nevertheless, this approach is hampered by high variance for irradiated samples and blanks and also by a significant number of low-sensitivity samples showing false negative results.

When the extraneous mineral grains on sample surfaces were separated from the organic bulk and the TL of the two phases was separately measured, TL sensitivity enhancements of 10³ or more and improved signal-to-blank ratios were seen in mineral phases (Fig. 1). These experiments clearly show that the TL is associated with the

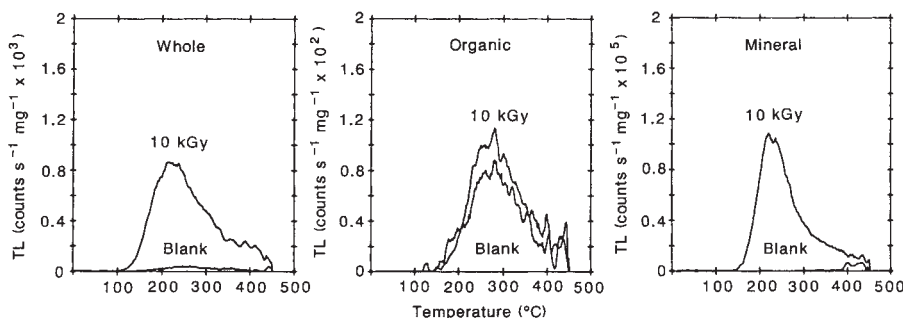


FIG. 1 Comparison of TL glow curves recorded for irradiated (10 kGy) and unirradiated (Blank) portions of whole sage and its organic or inorganic fractions. TL strength expressed as weight-normalized photon count rates obtained from an SURRC research reader incorporating an EMI 9883QB photomultiplier filtered with KG1 and 7/59 glass filters. Samples mounted on stainless steel disks (0.25-mm thick, 1-cm diameter) and heated in O₂-free N₂ at 6 °C s⁻¹. Minerals separated by ultrasonic agitation in solutions of sodium polytungstate (density 1.6 g cm⁻³) followed by centrifugation.

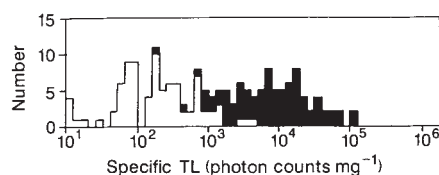
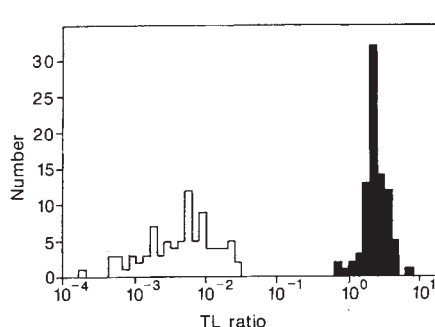


FIG. 2 Comparison of the discrimination between irradiated (black) and non-irradiated (white) whole samples (above) and mineral grains (right) of 85 herbs and spices. TL signals from mineral grains were normalized to a second (2.5-kGy) exposure.

mineral grains, even though they account for very little of the samples (less than 1 per cent by weight in Fig. 1). Investigations of pure organic compounds such as oleoresins have so far failed to identify strong radiation-induced signals. The small residual signals from the organic components are predominantly due to spurious TL, not induced by radiation.

Refined measurement procedures based on mineral grains have therefore been developed to improve discriminating power. The highly variable mineral yields and specific TL sensitivities from different samples posed problems in cross-contamination and in normalizing the sample response. Cross-contamination was overcome using rigorous blanking and handling procedures. Normalization was accomplished using a re-irradiation method, scaling the TL recorded in first



glow to that induced by a fixed dose (2.5 kGy) administered before a second measurement.

The results for a subset of 85 herb and spice samples are shown in Fig. 2. Measurements on separated mineral grains show a dramatic improvement in discrimination compared with measurements on whole samples. The mean TL signal from irradiated samples is 352 times that from blanks; the lowest signal from an irradiated sample is over 25 times higher than the highest blank. The stability of signals from selected samples under various storage conditions has been studied for more than two years, and will continue. Stable signals are available from parts of the glow curve for all samples. The same approach can be extended to vegetables and fruits.

The TL signals from herbs and spices have been clearly shown to originate from

small quantities of adhering mineral grains. Whereas measurements on whole samples may be appropriate for rapid screening — identifying irradiated samples with about 90 per cent confidence — measurements on separated mineral grains offer a far higher level of confidence. Given appropriate laboratory quality control and sample credentials, such measurements can be used as the basis of a reliable test for identifying irradiated foods.

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Continuum scaling for spreading drops

SIR—Heslot *et al.*¹ reported striking observations in the spreading of small droplets, which included evidence of discrete layers and a precursor film of a thickness comparable to the lateral dimension of a single molecule. This raises the question of whether continuum approaches for dealing with droplet spreading at these scales must be abandoned. It is salutary to consider a scaling argument.

If gravitational effects are assumed negligible, the spreading of the wetting droplet is driven by surface tension (σ) and opposed by viscous shear within the droplet. Taking the droplet radius (r) to

be defined by the shoulder of the precursor film, it follows that surface forces would scale roughly as σr . Viscous forces would scale as the product of viscosity (μ), shear rate ($(r/t)/h$, where h is film height and t the time elapsed) and droplet area (r^2). Mass conservation requires that droplet volume (V) scale as $r^2 h$, so then viscous forces would scale as $\mu r^3 V^{-1/3} t^{-1/3}$. A dominant balance of surface and viscous forces would thereby suggest that

$$r \approx k (\sigma V t / \mu)^{1/4} \quad (1)$$

where k is an empirically determined scaling constant presumed to be of the order of one.

The figure shows data for precursor-shoulder displacement as a function of time^{1,2} and the relationship indicated in equation (1) with the coefficient k taken as 0.5. Both with regard to the scale of lateral droplet dimension (in that k turns out to be about one) and its time dependence (close to $t^{1/4}$), spreading of the

precursor film is not inconsistent with the notion of continuum fluid flow.

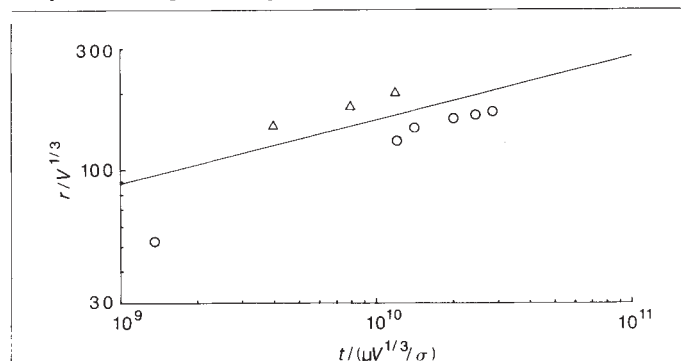
If we attribute the discrepancy evident for short times to the systematic change in droplet shape (and characteristic shear rate) seen to accompany early spreading, then the data suggest neither a finite equilibrium radius nor a departure from continuum scaling ($r^{1/4}$) as the droplet spreads, emergence of discrete layers notwithstanding. Extension of the continuum approach into a domain in which the discrete nature of the medium might be thought to dominate is a curious result but is not without precedent.

Of course, one should not draw too general a conclusion from the limited data available. Equation (1) suggests obvious generalizing approaches through which to test the idea more rigorously. If the continuum scaling law in equation (1) does apply to the spreading of small droplets, it would be interesting to seek empirical evidence that might demark its limits of applicability.

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Precursor-shoulder displacement as a function of time, from Fig. 2 of ref. 1 (circles) and Fig. 1 of ref. 2 (triangles). The solid line represents the relationship in equation (1), with $k = 0.5$.

Taking the high ground

Richard Dawkins

Arguments on Evolution: A Paleontologist's Perspective. By Antoni Hoffman. Oxford University Press: 1989. Pp. 274. £22.50, \$29.95.

"THIS is a book of unabashed criticism." Thus Hoffman begins his preface. I take the allusion, even if nobody else does. But if I had been writing the book, I'd have opened with a different reference:

Bring the balloon of the mind
That bellies and drags in the wind
Into its narrow shed.

Macroevolutionary studies in recent years have been dominated by what its opponents might call the windbag school of palaeontology. Now Antoni Hoffman hits back on behalf of what his opponents might call the killjoy school.

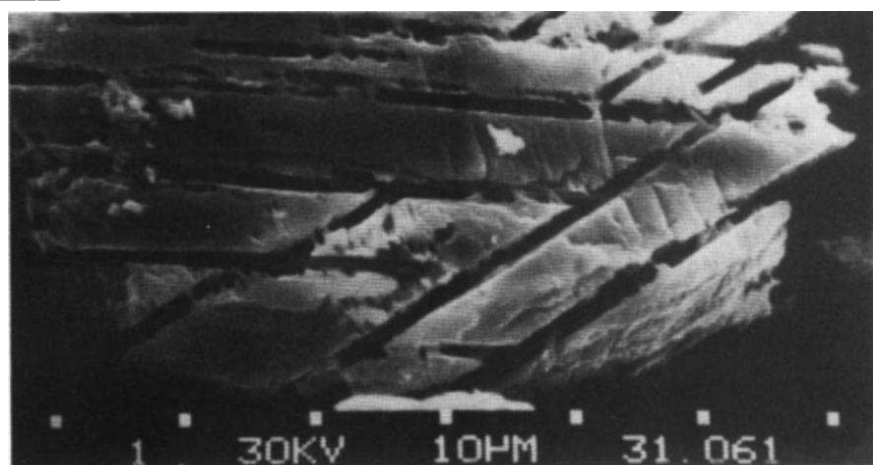
The windbag school gets its name, of course, from the inflation of levels of explanation. It is no great achievement to notice that life is organized in a hierarchy of levels. As a matter of convenience if nothing else, we use a different language when working at the different levels, and different kinds of people tend to work there. Among evolutionists macroevolution is the natural domain of palaeontologists, microevolution lies in the province of ecological geneticists. But is it just a difference of convenience or are there wholly different phenomena peculiar to the level of macroevolution? It had been the elegant conclusion of G.G. Simpson and his colleagues of the modern synthesis that macroevolution is simply what you get when you let microevolution go on for a sufficiently long time — which means a very very long time indeed. But others have wished to invoke wholly new explanatory principles for macroevolution, principles that cannot be derived by extrapolation from microevolution.

There are respectable precedents for invoking new kinds of explanation at higher levels. When we are trying to understand how certain kinds of complicated mechanisms work, for instance computers or nervous systems, it is the only way to proceed. It is true that computers work by changing the patterns of 1s and 0s (high and low voltage) in a large array of semiconductors, but when you've said that you haven't said anything very helpful. If you want to understand how your Macintosh actually achieves the feat of, say, shrinking a window, talk of intercutting 1s and 0s isn't going to help you much. You need a higher level of explanation, a 'toolbox' of software procedures calling other procedures down through a hierarchy. This is not wanton inflation of levels of explanation; it is a dire necessity.

But what is true for computers doesn't have to be true for evolution. Time is a continuum. Aeons really are no more than milliseconds laid end to end. It is entirely possible that the processes that operate on a timescale of years, if accumulated over a sufficiently large number of years, will add up to the processes that palaeontologists pick up as macroevolution. I find it a noble, awesome thought that this might be so, like the thought that the Scottish Highlands were once as high as the Alps but have been ground down by erosion too slow to be picked up during a human lifetime. But computers and brains are noble

targets: punctuated equilibria, species selection, the decoupling of macroevolution, large-scale patterns of diversification of life on Earth, rhythmical mass extinction, and Nemesis, the postulated sister star of the Sun. These are all fun to lecture on, and they make popular bite-sized undergraduate essay topics. But there is something noble, if austere, in the attempt to explain everything in terms of a very few basic principles working on different time scales. Conjuring new 'levels of explanation' out of hats all up and down the ladder of life seems, by contrast, unimaginative, fussily *ad hoc*.

But there we are, back with rhetoric. What finally matters is what is true. Hoffman is short on rhetoric, long on concern for truth. He is scrupulously fair to his opponents, in many cases giving a clearer and more fully worked-out account of their theories than they do themselves. This raises our confidence in his demolition when it comes. I am not claiming that



Evidence of impact — micrograph of a shocked-quartz grain from the iridium-rich layer at Brownie Butte, Montana. The picture is reproduced from *Mass Extinctions: Processes and Evidence*, edited by Stephen Donovan and published recently by Belhaven, London.

and awesome too. Is macroevolution, as a matter of fact, like a computer or like the Highlands? This is not a question that can be decided by rhetoric. A detailed, meticulous, intelligent, critical assessment of all the evidence is what we need, and that is what Hoffman gives us. A well-constituted and conscientious Royal Commission on the subject would produce a report very like Hoffman's book. His verdict is a decisive victory for the Highlands over the computer. 'Megaevolution' is what you get when macroevolution has been going on for sufficiently long, macroevolution is what you get when microevolution has been going on for sufficiently long, and microevolution is what you get when individual generations have been succeeding one another for sufficiently long.

So, is Hoffman really a killjoy? That depends on where you find your joy. It is true that he takes his balloon-humbling needle from one to another of a whole range of fashionable and charismatic

the last word has been said. Hoffman himself is careful to stress that his case is based only on the evidence at present available. He is not an ideologically committed, doctrinaire darwinian but a darwinian whose conviction comes from exhaustive examination of the evidence. Incidentally, although the idea of mass extinction is one of Hoffman's targets, there is nothing remotely anti-darwinian about mass-extinction, unlike species selection and 'decoupling'. I myself should not be surprised if Nemesis, or something equally catastrophic, one day returns to visit us. If it does, the extinction toll may include 90 per cent of all species on this Earth, but darwinism itself will not be a casualty. It will scarcely even pause before getting back down to the slow, uniformitarian grind and filling the vacuum. □

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Bruce Bohor and colleagues

PROTEIN-NUCLEIC ACID INTERACTION

Volume 10 Topics in Molecular and Structural Biology

Edited by W. Saenger and
U. Heinemann.

The book contains articles highlighting the rapidly evolving field of protein-nucleic acid interactions. Individual chapters demonstrate the diversity of biological systems to which protein-nucleic recognition is of central importance.

There are chapters on the interaction of small proteins with DNA and RNA as well as on chromatin organisation. Care has been taken to include accounts of many different experimental approaches to studying the subject. All articles are written by scientists actively involved in the research they describe and considered experts in their respective research areas. The book is well-illustrated and contains extensive bibliographic information to provide easy access to the original literature.

Contents DNA-Protein Interactions in the Regulation of Gene Expression - P.H. von Hippel (University of Oregon, Eugene, USA) and O.G. Berg (Uppsala University, Sweden). / Structures of Protein-Nucleic Acid Complexes in Solution by Electro-optical Analysis - D. Porschke and J. Antosiewicz (Max-Planck Inst., Göttingen, FRG). / NMR Studies of Protein-DNA Recognition. The Interaction of LAC Repressor Headpiece with Repressor DNA - R. Kaptein, R. Boelens and R.M.J.N. Lamerich (University of Utrecht, Holland). / The Single-stranded DNA Binding Protein of *Escherichia coli* - J. Greipel, C. Urbanke and G. Maass (Medizinische Hochschule, Hannover, FRG). / Protein-Nucleic Acid Interaction in Tobacco Mosaic Virus - G. Stubbs (Vanderbilt University, Nashville, USA). / Structural and Functional Studies of Ribonuclease T1 - U. Heinemann and U. Hahn (Freie Universität, Berlin, FRG). / Tet Repressor-Tet Operator Interaction - W. Hillen and A. Wissmann (Inst. Mikrobiol. and Biochem. Erlangen, FRG). / Structure and Condensation of Chromatin - M.H.J. Koch (DESY, Hamburg, FRG). / Conclusion / Index

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Grounds for agreement

John A. Endler

Analytical Biogeography: An Integrated Approach to the Study of Animal and Plant Distributions. Edited by Alan A. Myers and Paul S. Giller. Chapman & Hall: 1988. Pp. 578. £49.50, \$87.50.

BIOGEOGRAPHY is as basic to biology as evolution, but the subject's development has been hindered by three historical constraints. First, until the pioneering work of D. Rosen, MacArthur and Wilson, it had largely stagnated into data collection and narrative model-making. Second, the past 20 years have been so filled with dispute that it has been difficult for outsiders to take the subject seriously. And third, biogeography became so fragmented that schools representing different approaches ignored each others' work; it was essentially impossible, even in textbooks, to get an idea of the full range of the subject. *Analytical Biogeography* shows that things have changed, and that biogeography can be a fascinating, rigorous and unified subject.

The editors have done an admirable job of selecting subjects and authors. Only two topics have been missed — the use of statistical tests for non-random spatial patterns and specific biogeographical hypotheses (for example the Mantel test and spatial autocorrelation); and the use of unintentional and intentional introductions of organisms in experimental biogeography. Moreover, only a trace of the former animosity between different schools is evident. Although there are contradictions among the chapters, the authors have made a serious and usually successful effort to discuss both the strengths and weaknesses of their own approaches, and to refer to other chapters. In addition, the editors' introductions to each section put the individual contributions in a broad perspective.

The sections are arranged by biogeographical perspectives, patterns, processes and reconstruction. But the chapters more naturally fall into groups with common approaches in different time-scales: current and fossil evidence, static and dynamic interpretations, and ecological and historical processes. Biogeography aims to explain the distribution of organisms on all time-scales, past and present. But some contributors (Brown, Williamson, Parsons, T. Schoener, A. Schoener) concentrate on smaller time-scales (ecological), while others (Brundin, Humphries *et al.*, Craw) concentrate on larger ones (evolutionary, geological or historical). The respective aims and methods vary; they have different implica-

tions for the spatial scale of pattern and the processes which affect the maintenance, changes in and origins of geographical distributions. Studies of small time-scale patterns and processes tend to concentrate on extant species, static (equilibrium) phenomena and ecological explanations, while those of large time-scale patterns tend to emphasize time-dependent processes, and geological, phylogenetic and evolutionary interpretations. Although small time-scale phenomena are potentially observable and subject to experimentation, they cannot directly address long-term phenomena; large time-scale studies can make use of both extant and fossil data (see especially B. Rosen's chapters), but can never be experimental. Both extremes have their strengths and weaknesses, so the most productive approaches use several time-scales and consider equally all possible processes (Myers and Giller, B. Rosen, Major, Marshall, Lynch).

One of the biggest problems is how to assess the relative importance of historical and ecological factors (Myers and Giller, B. Rosen, Lynch). This is not merely a matter of scale, because long time-scales allow time for convergent evolution, which can distort or obscure the effects of historical processes. For example, Craw discusses Croizat's proposal, based upon panbiogeography of vegetative morphology, of a novel close relationship between three carnivorous plant families. The analysis yields a transformation series linking the families' floral morphology, which is incongruent with vegetative morphology. But their vegetative morphology functions in trapping insects in habitats with low soil nutrients; the transformation series is distorted by convergent evolution driven by common ecological conditions. A converse example is that sympatric plant species often have divergent leaf morphology which may result from historical rather than ecological processes.

A key problem in the foregoing studies, and throughout biogeography, is the dearth of specific predictions for each of many possible hypotheses. In fact it was the few specific hypotheses which set the separate biogeographical schools on their way. *Analytical Biogeography* makes the gaps explicit and comprehensible for the first time. □

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• Eugene P. Odum has written what he himself calls a hybrid volume — in part a revision of his textbook *Ecology*, in part "a citizen's guide" to the principles of the subject. Entitled *Ecology and Our Endangered Life-Support Systems*, the book is published by Sinauer Associates, Sunderland, Massachusetts, and distributed in Britain by W.H. Freeman. Price in pbk \$14.95, £10.95.

From lamps to lasers

Willem D. Hackmann

The GEC Research Laboratories 1919–1984. By Robert Clayton and Joan Algar. *Peter Peregrinus/Institution of Electrical Engineers: 1989. Pp.438. £40. Available in the US from IEEE Service Center, 445 Hoes Lane, Piscataway, New Jersey 08855, USA, price \$80.*

THE oldest British government-sponsored laboratory was the Royal Observatory at Greenwich, established by Charles II to improve navigation. As the ensuing decades turned into centuries, government agencies came increasingly to rely on scientific expertise, the eventual result of which was the creation of the National Physical Laboratory (NPL) in 1897. This establishment formed the pattern for subsequent state and commercial laboratories in Britain (now, in the case of state laboratories, being reversed), but American and German firms were quicker off the mark in creating such facilities. Thomas Edison's establishment, first at Menlo Park (1876) and then at West Orange, New Jersey (1887), was the largest privately funded laboratory in the world at that time, and epitomized the transition from the nineteenth-century lone inventor-entrepreneur to organized twentieth-century industrial research, creating new products and markets.

There is scant information on the early development of commercial research laboratories in Britain, in part because many firms have destroyed their archives. Robert Clayton and Joan Algar have produced a densely written book about one of Britain's best-known commercial research centres, the General Electric Company Research Laboratories at Wembley (later called the GEC Hirst Research Centre after the company's chairman and managing director who founded the laboratory). It must be said, though, that this is not so much a history as a chronology of events, concentrating on scientific and organizational developments and not on people. The only personality of which we get a glimpse is the first director, Sir Clifford Paterson, a somewhat austere figure with distinctive views on research.

During the First World War, Paterson was working at the NPL in charge of the Electrotechnics Department and Photometry, with contacts at the Osram Lamp Works (then a separate company founded jointly by the DGA of Berlin and GEC of England). Thus it was natural that he should be approached by GEC and Osram to give advice on the setting-up of a research facility, so that the English

company would be independent of German science. Indeed the First World War had a great effect on English industrial and government research, which had to be re-organized to make up for the loss of German technical products.

In the event, Paterson undertook the task of building up the GEC Research Laboratories himself. The facility was opened in 1923 by Sir J. J. Thomson, the Cavendish professor of experimental physics at Cambridge and Master of Trinity College, and the Member of Parliament, Lord Robert Cecil. Even by the standards of the period, Paterson's ideas were unorthodox: the administration was kept to a minimum, the main structure of the research organization remained in the hands of scientists, and the commercial management of GEC did not interfere with the scientific work. Paterson held strong views, not least on punctuality. When, during the war, one employee blamed bad time-keeping on a broken alarm clock, Paterson provided a new one and duly debited the cost at the end of the following month. The centre's organization only started to change drastically after Paterson's death in 1948.

The authors have made little attempt to place the developments at Wembley in a wider context, except when they are related to specific research elsewhere. The first two chapters are the most historical. After dealing with the "origins, philosophy and organisation" of the laboratory, a synoptic outline is given of the main research work by decade, from the 1920s until 1984. This account is amplified in the next 14 chapters on work done by the various sections of the laboratory, written by specialists from these sections. Thus we have chapters on lamps and

valves (the initial stimulus for the laboratory's existence), cathode-ray tubes, communications and electronics, semiconductors, physics (mostly solid-state), lasers and optoelectronics, glass and refractory materials, metallurgy, and chemistry.

Next follows an entire chapter devoted to research during the Second World War, which, although carried out for the most worthy patriotic sentiments, stood GEC in good stead in the early post-war period. Highlights are GEC's contribution to magnetron research (starting with the glass-envelope magnetrons of the early 1930s), the development of the Randall-Boot cavity magnetron (which was handed over to the United States by the Tizard Mission in 1940), airborne radar for fighter planes, radar for gun-laying and 'blind-bombing', and acoustic torpedoes. The final, brief chapters deal with statistics and quality control (GEC was a pioneer in the application of statistical methods), patents and publications.

The authors point out that this history is almost entirely based on first-hand accounts, primarily technical reports. The book itself is cast in the style of a scientific monograph, giving fascinating insights into the workings of an industrial laboratory and showing in microcosm what has happened in general in British commercial research during the twentieth century. In particular, one is struck by the fact that the backbone of commercial research is based not on epoch-making discoveries or on flashes of genius, but on careful, meticulous work leading to steady progress. □

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In the back room: work on television at GEC after the Second World War.

Designs for living

Paul Crowther

What Is Art For? By Ellen Dissanayake. University of Washington Press: 1988. Pp.249. \$20. Available in the United Kingdom from Trevor Brown Associates.

QUESTIONS about the nature and function of art have traditionally been regarded as the special province of the aesthetician and art historian. Aestheticians, for example, have frequently sought to define art on the basis of phenomenal properties held in common by all artworks. But although they have indeed clarified the complexity of the problem, they have not, as yet, offered anything like a definitive solution.

Dissanayake's project, then, is to discover whether that which is essential and distinctive to art might be more accessible to definition on the basis of a 'biobehavioural', ethological approach — that is, one which asks questions such as 'What is art for?' and 'What role does it play in the evolution of our species?'. That art does have such a role is, at first sight, tenable — for despite enormous differences in patterns of practice or execution, at least one or other of the arts (be it dancing, singing, carving, decorating, music-making or versifying) is found universally in every known human grouping, present or past. So there are good grounds for postulating an 'evolutionary reason' for its persistence and ubiquity.

Dissanayake explores this idea by discussing various art practices in contexts ranging from analogues in the animal world, and their function in the psychological development of children, to their social role in palaeolithic and 'primitive' societies and their aesthetic significance in the modern world. In particular, she carefully acknowledges and avoids the problems of a Eurocentric bias, which would favour emphasizing art's aesthetic function over its many other roles.

The result of this approach is the claim that what characterizes behaviour as artistic is the practice or recognition of 'making special'. This is not mere making or creating. As Dissanayake puts it: "Making special implies intent or deliberativeness. When *shaping* or giving artistic expression to an idea, or *embellishing* an object, or recognizing that an idea or object is artistic, one gives (or acknowledges) a specialness that without one's activity or regard would not exist. Moreover, one intends by making special to *place the activity or artefact in a 'realm' different from the everyday*". The ethological significance of this practice is that when it is allied to 'life-serving' activities such as

ceremony or tool-making, it consolidates and reinforces their value in a way which enhances 'survivorship'.

The odd thing about Dissanayake's discussion is that it does not solve the problem which she takes herself to be addressing at the start of her book — that of providing a compelling definition of art. The reason is that although 'making special' is (on her terms) a necessary condition of art, it is not a sufficient one; play and ritual also share the same quality. She herself faults the rival approach of philosophical aesthetics for (among other things) attempting to define art in terms of features which turn out to be shared by things besides art. Yet that is just where Dissanayake's own

argument breaks down.

Despite this and some other philosophical worries about the book, Dissanayake's contention that art has ethological significance is itself plausible. Indeed, *What Is Art For?* is probably one of the most intellectually enriching interdisciplinary studies of art that has ever been written. It combines breadth of learning with lucidity of thought and expression, in a way that should engage the interest of anyone with a systematic interest in the arts — be it scientific, or from the viewpoint of the humanities. □

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Full of energy

A.W. Wolfendale

Cosmic Rays: Tracking Particles from Outer Space. By Michael W. Friedlander. Harvard University Press: 1989. Pp.160. \$27.50, £21.95.

OUR information about the cosmos comes from studies of both matter and radiation, the first being in the form of meteorites and the mis-named cosmic radiation, that rain of high-energy nuclear particles beating down on the top of the Earth's atmosphere. *Cosmic Rays* is a semi-popular account of some of the more important features of these enigmatic particles.

Friedlander's historical introduction is very good, as are his discussions of the relationship of the subject to the fields of high-energy physics and geophysics. The attempt to describe the link with astrophysics proper is disappointing, though, particularly with respect to the outstanding problem of cosmic-ray origin. We are led to believe, from the publisher's blurb and list of contents, that a serious effort will be made to tackle this question; unfortunately, it falls short of expectations.

The most dramatic feature of cosmic rays is the presence of particles as high in energy as 10^{20} eV. The origin of these particles is of the utmost interest, but this area receives very sketchy treatment. A dated spectrum shows no features from 10^{16} to 10^{20} eV, whereas it has been known for some years that there is structure in this range. Mention is made of "a suggestion" of a change in spectral shape above 10^{19} eV, but what was needed was a detailed treatment of this region. What are the directions of the particles? If extragalactic in origin, where have they come from? How do they propagate through the ubiquitous microwave background radiation in extragalactic space? These are some of the questions that should have been posed.

Turning to lower energies, the possibility of particles being accelerated by supernova shocks is mentioned, but it would have been useful to see the evidence set out. Indeed, gamma-ray astronomy — a growth area of cosmic-ray physics — receives scant mention, which is a pity because many of the clues for the origin of the bulk of cosmic rays are coming from this field.

This lack of sensitivity for contemporary astrophysics leads Friedlander to write in his penultimate paragraph.

... the history of science has shown how research fields flourish and then dwindle as new fields emerge. It may be that the contributions of cosmic ray research have for the present been exhausted. Those of us who have had the good fortune to participate in this research have had so much enjoyment that we should not begrudge others the excitement of seeing their fields prosper and move ahead.

I have news for the author. Cosmic-ray physics is alive and well. It is to be found in the giant extensive air-shower arrays running and under construction; in the great neutrino detectors; in the gamma-ray observatory to be launched into orbit later this year; and in the sophisticated detectors being designed to measure the isotopic composition of the cosmic rays and to search for particles of anti-matter in experiments in space and elsewhere. □

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New in paperback

- *The Cambridge Guide to the Material World* by Rodney Cotterill (Cambridge University Press, £14.95, \$27.95 — a snip at the price). For review see *Nature* **314**, 686 (1985).
- *Sewall Wright and Evolutionary Biology* by William B. Provine (University of Chicago Press, \$18.95, £14.25). For review see *Nature*, **324**, 175 (1986).
- *Darkness at Night: A Riddle of the Universe* by Edward Harrison (Harvard University Press, \$12.95, £10.25). For review see *Nature* **330**, 288 (1987).
- *Statistics for Nuclear and Particle Physicists* by Louis Lyons (Cambridge University Press, £10.95, \$19.95). For review see *Nature* **325**, 586 (1987).

Upper limits on neutron and γ -ray emission from cold fusion

M. Gai*, S. L. Rugari*, R. H. France*, B. J. Lund*, Z. Zhao*, A. J. Davenport†, H. S. Isaacs† & K. G. Lynn‡

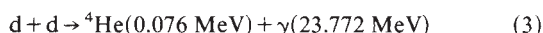
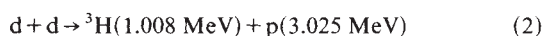
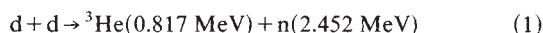
* A. W. Wright Nuclear Structure Laboratory, Yale University, New Haven, Connecticut 06511, USA

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Neutron and γ -ray emission from a variety of electrochemical cells (running continuously for up to two weeks) have been measured using a sensitive detection system with a very low background. Titanium alloy powder deuterided at room temperature and high pressure was also used for comparison. No statistically significant deviation from the background was observed in either γ -ray or neutron detectors. The estimated neutron flux in this experiment is at least a factor of 50 times smaller than that reported by Jones *et al.* and about one million times smaller than that reported by Fleischmann *et al.* The results suggest that a significant fraction of the observed neutron events are associated with cosmic rays.

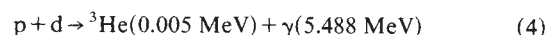
It has been suggested recently^{1,2} that deuterium in hydride-forming metals (palladium and titanium) can undergo nuclear fusion at room temperatures to release heat, neutrons, tritium and helium. Deuterium was incorporated into the metal lattices by electrolytic charging in solutions of heavy water. The nuclear reactions between deuterium nuclei that are energetically feasible (the 'open channels') are as follows:



Reactions (1) and (2) take place at similar rates³, as suggested by room-temperature muon-catalysed fusion in molecular

hydrogen¹ whereas reaction (3) can be assumed to be 4 to 5 orders of magnitude slower. Nuclear-fusion events must be characterized by the production of the ionizing radiations listed above, as there is no known mechanism that inhibits their emission.

It has been suggested that interactions between protons and deuterons,

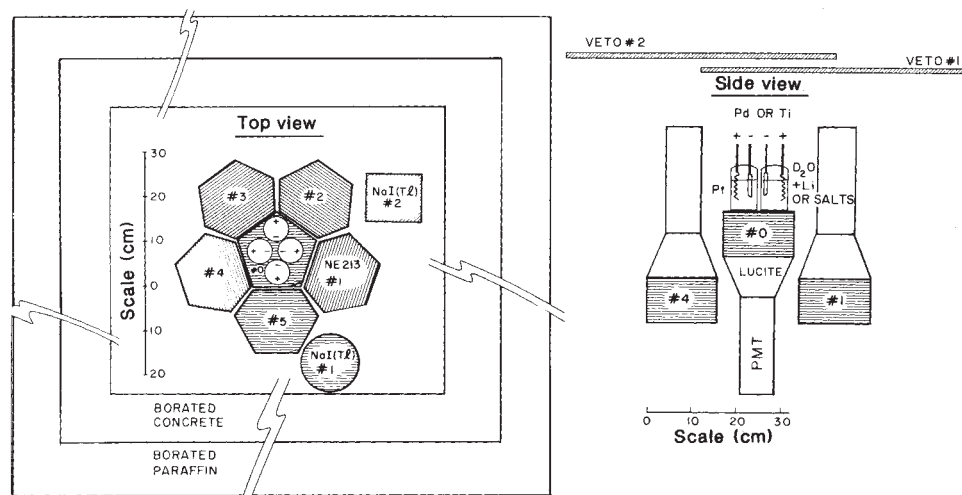


could also take place (at a faster rate) in metals charged with deuterium (and hydrogen) from heavy water (refs 4, 5 and S. E. Koonin, personal communication). Even if the hydrogen content in heavy water is small, significant concentration of hydrogen in the metal can take place because the rate of hydrogen-ion reduction on the cathode surface is greater than that of deuterium ions by up to a factor of 13 (ref. 6), and the diffusion rate of deuterium in Pd is only a factor of 2 larger than that of hydrogen⁷.

Jones *et al.*¹ reported that, in their cells, neutrons from reaction (1) are detected at a small average rate of 2 counts h^{-1} above background. In one experiment (run 6) they report a rate of 15 counts h^{-1} for neutrons of energy $2.5 \pm 1 \text{ MeV}$. With neutron detection efficiency of 1% and using a Ti electrode of mass 3 g, they quote a cold fusion rate for $d + d$ of $\lambda_f \approx 10^{-23}$ fusions per deuteron pair per second (ref. 1). Fleischmann *et al.*² estimated a larger neutron flux of 4×10^4 neutrons per second from a 15-g Pd rod, measured with a neutron dosimeter with a detection efficiency of 2.4×10^{-6} .

Here we report on a search for neutrons and γ -rays emitted during conjectured fusion of $d + d$ and $p + d$ in various metals into which deuterium has been incorporated ('deuterided' metals). Measurements were carried out with an array of low-background, high-sensitivity neutron detectors and two large high-efficiency NaI(Tl) γ -ray detectors. The contribution from

FIG. 1 Schematic diagram of the experimental setup, drawn to scale.



cosmic rays was measured with two large-area cosmic-ray veto counters. Four cells were used concurrently, with electrodes and solutions based on those used in the experiments reported by Jones *et al.*¹ and Fleischmann *et al.*². In addition, one Ti alloy powder sample deuterided by gas-phase deuterium was used (see Table 1 footnote). We report here on data taken over a period of three weeks. We observe no statistically significant excess of counts of either neutrons or γ -rays above the background, and we have obtained upper limits on the rates of cold fusion in deuterided palladium and titanium.

The detector system

The neutron detector system is shown in Fig. 1. It consists of six NE213 liquid-scintillator neutron counters with fast-photo-multiplier tubes selected from the Yale neutron ball⁸. Each detector was 10 cm thick. The central detector (number 0) was a regular pentagon (inscribed in a circle of radius 9 cm). The four electrolytic cells were placed on this detector with the palladium or titanium cathodes close to the centre, for maximum efficiency of neutron counting. The other five detectors (numbers 1 to 5) were irregular hexagons with dimensions similar to that of detector 0. These were arranged in a ring displaced 15 cm below detector 0.

State-of-the-art pulse-shape-discriminator electronic modules (ref. 9; modules produced by W. F. Piel) were used to distinguish between neutrons and γ -rays. A hardware gate was used to discard almost all detected γ -ray events in each detector. The efficiency of the neutron detectors was measured with calibrated neutron sources: ^{252}Cf (neutron energy $E_n = 2 \pm 1$ MeV) and Pu-Be ($E_n = 4 \pm 2$ MeV)¹⁰ placed in the middle of the four cells above the centre of detector 0. The efficiency is sensitive to the value of the threshold, which was set at ~ 80 –100 keV (electron equivalent), corresponding to neutron energies of 400–500 keV (ref. 11). The average efficiency of the detectors was measured as $\sim 25\%$ for neutrons from the ^{252}Cf source, in agreement with the value calculated from a simple model¹¹. The efficiency of individual detectors ranged between 16% and 36%, reflecting individual thresholds and detector performance. The possibility that the detectors were only sensitive to the higher-energy neutron tail of the ^{252}Cf source can be excluded, as the spectrum includes less than 7% of neutrons with energies in excess of 2.5 MeV (ref. 10).

The detection of neutrons is characterized as a coincidence event between a primary neutron registered in detector 0 and a scattered neutron (with approximately half the energy of the primary neutron, as a result of detector geometry) detected in one of the detectors 1 to 5. A neutron event is then defined by measuring its pulse shape in the second detector, the pulse heights in the two detectors, and the time of flight between the two detectors, which is related to the energy of the neutron. The coincidence method significantly lowers the background in the detector system and at the same time yields a reasonable efficiency (discussed below). The time differences (t) between successive events in detector 0 and any one of the detectors 1 to 5, for neutrons from a Pu-Be neutron source, are shown in Fig. 2a, and from a ^{252}Cf neutron source in Fig. 2b. The raw data include γ -ray (γ - γ') and neutron scattering events (n - n'). Gamma-ray-neutron coincidences (γ - n) are also evident in the spectrum. The γ - γ' time resolution was measured, using standard γ -ray sources (^{60}Co , ^{133}Ba and ^{137}Cs), to be ~ 2.5 ns; an appreciable contribution to the observed time resolution comes from 'electronics walk' as a result of the low threshold. In Fig. 2c we show the time spectrum gated by the neutron pulse shape of detectors 1–5. We are clearly able to remove γ - γ' coincidence events in the source data (these appear in Fig. 2b) by using this software gate on the pulse shape, without significantly affecting the n - n' coincidence events. The time of flight between detectors whose centres are 20 cm apart can be estimated on the assumption that the scattered neutron emerges with 50% of the energy of the primary neutron. The value expected for a 1-MeV neutron (^{252}Cf source) is $\Delta t = 14$ ns and for a 2-MeV neutron (Pu-Be source) is $\Delta t = 10$ ns. These are in approximate agreement with the centroid separation of the n - n' and γ - γ' peaks shown in Fig. 2.

The total efficiency of the neutron detection system in n - n' coincidence mode was measured using a ^{252}Cf source to be $1.0 \pm 0.2\%$ which is similar to that of Jones *et al.*¹. Neutron-neutron coincidences from two primary neutrons from the ^{252}Cf source (emitting 3–4 neutrons per fission) are suppressed by a factor of 16 because of the small solid angle over which measurements are made, and thus do not affect our measured efficiency. This value of the efficiency is consistent with a solid angle of 30% of 4π for detector 0, a solid angle of 45% of 2π for the ring of detectors 1–5 and an average intrinsic efficiency of 25%

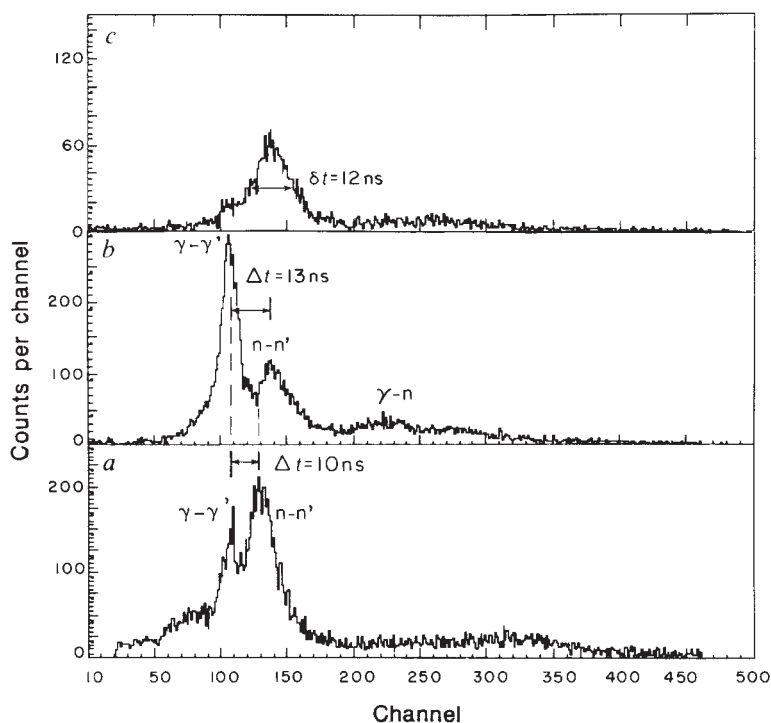


FIG. 2 Typical time-of-flight spectra measured with a, Pu-Be, and b, ^{252}Cf neutron sources, and c, with a cut on the neutron pulse shape, for the ^{252}Cf data. The time calibration is 2.5 channels per ns, with $t=0$ defined at the location of the p - p' peak. In a and b, Δt is the neutron time of flight. In c, δt is the width of the neutron time-of-flight distribution.

for the individual detectors. Calibration spectra were measured every 1–2 days to ensure that no drifts occurred in the system.

The neutron counting rate in each detector was ~ 120 counts h^{-1} over the entire energy range of the detector (for neutrons of energy < 6 MeV). In the coincidence-time spectra the rates are less than 1 count h^{-1} in each pair of detectors and, on average, 1.6 counts h^{-1} in the entire array of all five pairs. All five time spectra between detectors 0 and 1–5 were summed at the end of each run. The neutron events are summed in a region extending from the γ - γ' peak (defining time of flight $t \approx 0$ ns) to $t = 40$ ns (that is, 100 channels beyond this peak). The latter boundary corresponds to the largest flight time possible for neutrons of lowest energy (400–500 keV, that is, just above the detection threshold).

Two large-volume 12.5×12.5 cm NaI(Tl) detectors, with a resolution of 7% at 1.33 MeV and total full-energy peak efficiency (for each detector) of 0.1% at 5.5 MeV, viewed the cells directly at a distance of 32 cm, as shown in Fig. 1. Two large-area, 6-mm-thick, plastic-scintillator veto-counters, with dimensions of 120×60 cm and placed 35 cm above detector 0, were used to detect energetic cosmic rays traversing the apparatus, with a veto gate extending for 3 μs . These detectors subtended an angle of $\pm 60^\circ$ above the samples, with a veto efficiency of about 65%. The veto efficiency was measured using the high-energy portion (31-MeV) of the NaI(Tl) spectra, as shown in Fig. 3. In this region the count-rate is due to cosmic-ray interactions and the veto efficiency is given by the ratio of the two rates (Fig. 3).

Data were collected event-by-event on a Concurrent 3230 computer and stored on tape for later analysis. Each recorded event includes up to 20 parameters. Pulse-shape and pulse-height data were recorded using two 8-channel ORTEC AD811 analog-to-digital converters, and time-of-flight data were recorded using an 8-channel LECROY 2228A time-to-digital converter. The data collected during runs 8–14 were accumulated using a CAMAC crate interfaced to an IBM PC/AT compatible (20 MHz) Dell 310 computer with histogramming capability only.

Data were taken in two-hour run periods which were recorded and examined individually before being summed. Background was measured under various conditions: with cells removed from the shielded area containing the detectors (run 2), with cells in their normal position but passing no current (run 5), and with a cell including heavy water and LiI salt without electrodes (run 4). This last cell was used to assess the effects of cosmic rays on cells that included material with a large atomic weight.

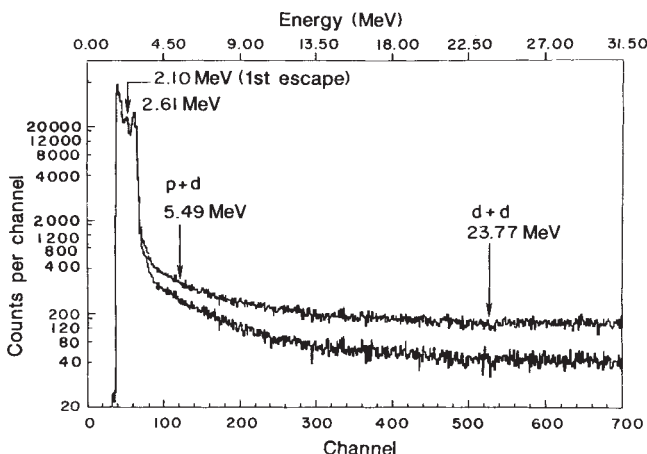


FIG. 3 Typical spectra from NaI(Tl) γ -ray detector 2 (run 6). The locations of the expected γ -rays from reactions listed in text are indicated. The raw γ -ray counts before and after vetoing on cosmic rays correspond to the upper and lower curves respectively.

The detector system was enclosed in a shielded housing of 15-cm-thick borated concrete and 15-cm-thick borated paraffin, and was covered with two layers of 15-cm-thick borated paraffin blocks. No lead shielding was used as this was found to increase the background. The housing's outer dimensions were $150 \times 150 \times 150$ cm. The experiment was carried out in a target area of the A. W. Wright Nuclear Structure Laboratory (with the tandem accelerator not running) under a 90-cm-thick roof composed of steel-reinforced concrete.

In the following we outline the procedures used in applying cuts on the data. Run 7 contains data from cell number 1 (running for 15 days), cells 2–4 (running for 1–4 days), and sample 9 (see Table 1) on the detector. In Fig. 4a we show the raw data as registered in the first channel of the time-to-digital

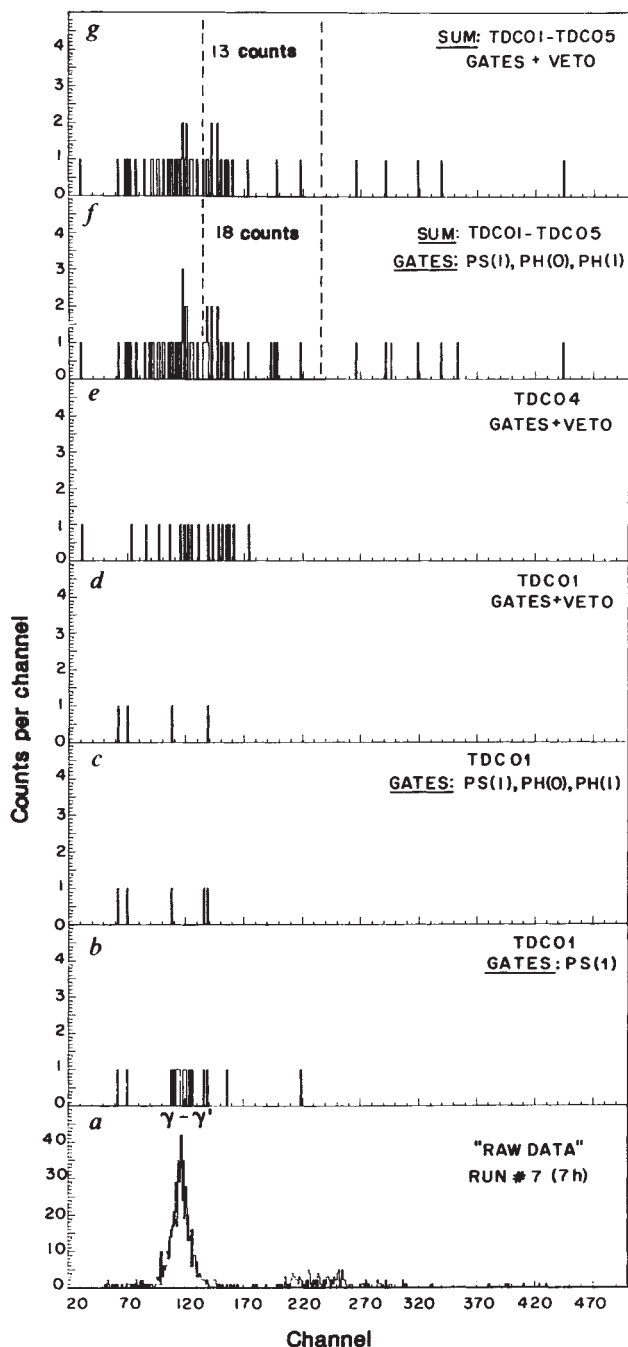


FIG. 4 Time spectra obtained during run 7 with cells on, with various gates and veto conditions as indicated, and discussed in text. The time range of interest, which brackets maximum and minimum times of flight of neutrons of about 2.5 MeV (discussed in text) is indicated by the vertical dashed lines.

TABLE 1 Measurements and elapsed times for charging electrodes (5–26 May 1989)

Run	Electrodes	Electrolytes	Charging time (h)	Counting time (h)	Run	Electrodes	Electrolytes	Charging time (h)	Counting time (h)
0*	1	3	up to 192	up to 60	8#	1	3	346	10.0
	2	5	up to 72	up to 60		3	1	155	10.0
	5	3	up to 72	up to 60		7	3	155	10.0
	6	5	up to 72	up to 60		4	2	77	10.0
1	1	3	192	20.3	9	1	3	356	7.2
	3	1		20.3		3	1	165	7.2
	7	3		20.3		7	3	165	7.2
	2	4	24	20.3		4	2	87	7.2
2†	1	3	216	9.7	10	1	3	364	17.3
	3	1	24	9.7		3	1	173	17.3
	7	3	24	9.7		7	3	173	17.3
	2	4	47	9.7		4	2	95	17.3
3‡	1	3	226	15.3	11	1	3	382	8.5
	3	1	34	15.3		3	1	191	8.5
	7	3	34	15.3		7	3	191	8.5
	8	5		15.3		4	2	113	8.5
	9				12	1	3	391	11.9
4§	None	10		6.1		3	1	200	11.9
5	4	2		11.9		7	3	200	11.9
6	1	3	270			4	9	122	11.9
	3	1	78		13**	1	3	411	23.5
	7	3	78			3	1	220	23.5
	4	2		28.6		7	3	220	23.5
7¶	1	3	299	7.0		4	9	142	23.5
	3	1	107	7.0	14	1	3	435	21.4
	7	3	107	7.0		3	1	244	21.4
	4	2	29	7.0		7	3	244	21.4
	9					4	9	166	21.4

ELECTRODES. (1) Pd plate 1×5×50 mm, cold-worked and heated in D₂ at 120 p.s.i. and 300 °C, and furnace-cooled under pressure (D/Pd=0.73), immersed to 3.1 cm (active area=3.7 cm²), anodized at 1 mA for 10 min. Electrical connections made with spot-welded Type 308 stainless steel rod, 3 mm in diameter. (2) Pd cylinder, 2 mm diameter, 100 mm long, annealed in flowing argon at 1,000 °C, immersed to 3.1 cm (active area=1.9 cm²), anodized at 1 mA for 10 min. (3) Pd cylinder, 6 mm diameter, 50 mm long, annealed in flowing argon at 1,000 °C, exposed to 120 p.s.i. D₂, anodized at 1 mA for 10 min., immersed to 3 cm (active area=5.8 cm²). Electrical connections as in (1). (4) Pd cylinder, 6 mm diameter, 50 mm long, with reduced section 2 mm diameter for electrical connection, annealed in vacuum at 900 °C, anodized at 50 mA for 10 min, immersed to 3 cm (active area=5.8 cm²). (5)–(8) Ti parallelepiped 3×3×45 mm, cold-worked, immersed 3 cm (active area=2.8 cm²). Electrical connections as in (1). (9) TiFe_{0.7}Mn_{0.2} powder (43 g) hydrided at 120 p.s.i. D₂, at room temperature, charged on 19 December 1987 and recharged on 4 April 1989. Contained in a 2 cm×20 cm cylinder. Etch for Ti: 5% HF (48%) in H₂O₂ (10 vol). Etch for Pd: 40% HNO₃ (98%): 60% HCl (36.5%).

ELECTROLYTES. (1) 0.1M LiOD/97.5% D₂O. (2) 0.1M LiOD/99.8% D₂O. (3) 1M LiOD/97.5% D₂O. (4) 1M ⁹LiOD/97.5% D₂O. (5)–(8) 100 g 97.5% D₂O + 0.125 g FeSO₄·7H₂O, 0.125 g NiCl₂·6H₂O, 0.125 g PdCl₂, 0.125 g Li₂SO₄·H₂O, 0.125 g Ti(SO₄)₂·9H₂O, 0.35 g CaSO₄, 0.11 g NaH₂PO₄, 0.125 g CaCO₃ adjusted to pH<3 with HNO₃. (9) 0.1M LiOD/99.3% D₂O. (10) LiI solution (30 g) in 100 cm³ heavy water. No electrode used.

* While experimental setup was improved, cells were on and counting was carried out with a reduced detector system. In this period five experiments were performed with the above electrode etched and abraded.

† Cells were moved out of shielded counting system, but cells remained on in a second, well shielded housing. Calibration measured.

‡ Cells were moved back into shielding together with sample 9, electrode 2 was replaced with 8. Calibration measured, cells on continuously.

§ Cells were run with electrodes 1, 3 and 7 outside of counting area. Calibration and background measured. Cells on continuously.

|| Cell off.

¶ Three cells returned to counting area, together with sample 9. Calibration measured, cells on continuously.

Counting system reduced to include only TAC01, PSD0 and NaI₂. Read by an IBM PC/AT compatible.

** Sample of heavy water (5 cm³) was taken for analysis from cells 1 and 4.

converter (TDC01), corresponding to the time of flight between two detectors, measured between a (common) start signal from detector 0 and a stop signal from detector 1. In these data we again observe the γ - γ' peak (defining time $t \approx 0$). Neutrons are expected in up to 100 channels beyond this peak, as discussed above. The background signal around channel 250 is due to the low threshold used in this experiment and arises from γ - γ' events. By using a software gate on the neutron pulse shape of detector 1 such that only neutrons are detected in this detector, we get the data shown in Fig. 4b, and by excluding neutrons of energies larger than 3 MeV, we get the spectra shown in Fig. 4c. This yields a neutron detection rate in the 0–1 pair of 3 counts in 7 h (note that these counts cannot all be resolved by eye in Fig. 4). The cut on higher-energy neutrons removes n-n' scattering events in the vicinity of the γ - γ' peak ($t \approx 0$), because higher-energy neutrons are fast. By vetoing of cosmic-ray events we are left with 2 neutron events in the region of interest (corresponding to the largest and smallest possible flight times,

and shown by dashed lines in Fig. 4f, g) after 7 h of counting in the 0–1 neutron detector pair (Fig. 4d). The 0–4 neutron-detector pair generally showed a higher counting rate (because of its larger measured efficiency), yielding 6 neutron events in 7 h (Fig. 4e). In the whole array, we obtained 13 counts for 2.5-MeV primary neutrons (Fig. 4g) in the area of interest, giving a neutron rate of 1.8 ± 0.4 counts h⁻¹.

Electrochemical procedures

The electrochemical cells used in this experiment were contained in 100-ml polyethylene bottles. The top of each bottle held the cathode (palladium or titanium, more than 99.9% pure), the anode (several strands of 0.5-mm-diameter platinum wire in an S-shape), a fine tube for the introduction of nitrogen gas presaturated with heavy water, and a vent hole for the nitrogen mixed with the gaseous electrolysis products. The flow rate of nitrogen through the cell was sufficient to dilute the deuterium gas to a concentration below 5%, thus reducing the risk of explosion.

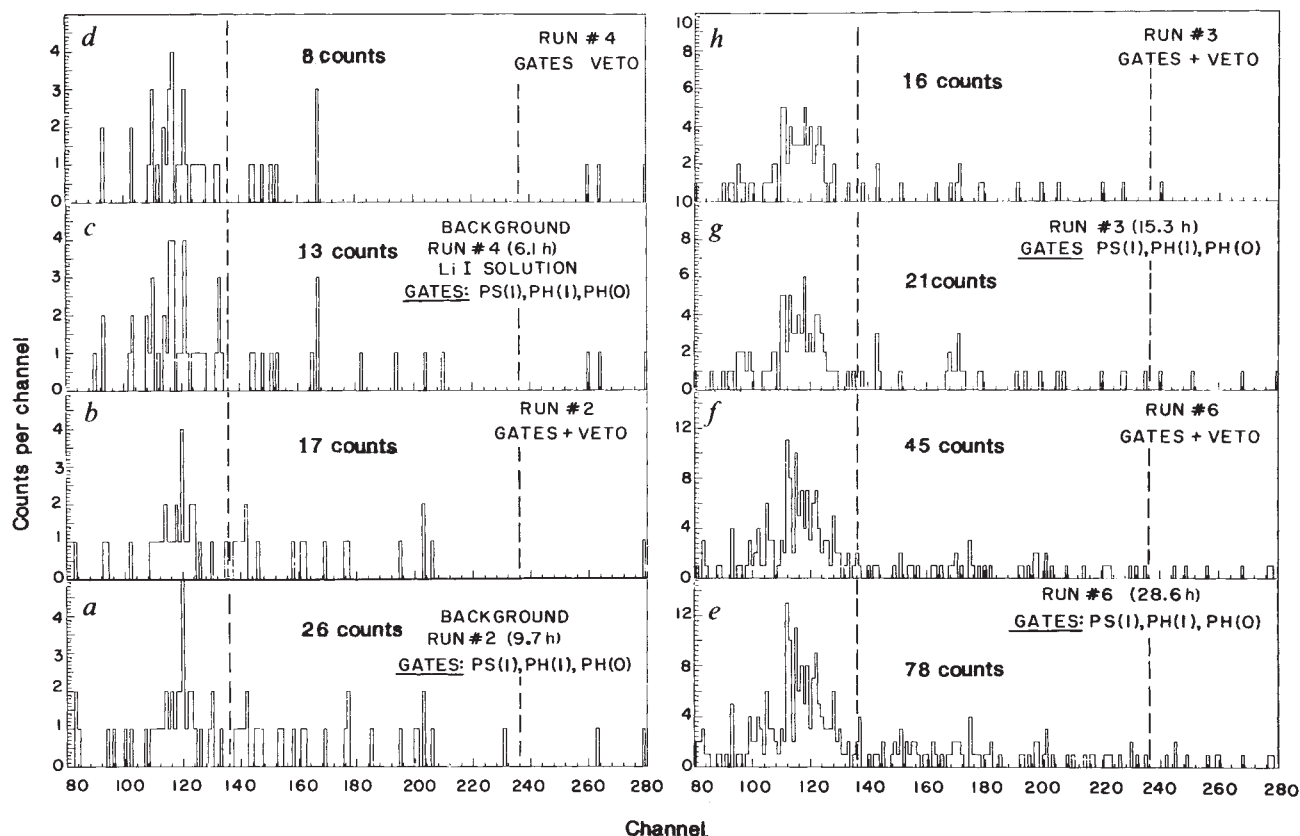


FIG. 5 Time-of-flight spectra obtained for various runs, both with cells on and off (background runs). The data in *g* were obtained under conditions

similar to those in ref. 1. For further details, see text.

The connections, dimensions and pre-treatments of the palladium and titanium cathodes are listed in Table 1, and included etching and electrode treatments. Two types of electrolyte were used: LiOD (ref. 2) and a complex mixture of salts with an acidic pH, based on that used in ref. 1. LiOD solutions were made by adding lithium metal to D_2O . The compositions of the different solutions used, and the 'cell-on' and counting times, are listed in Table 1.

A constant current was provided by PAR 363 potentiostat-galvanostats. A current of 300 mA was used for all the cells except that including electrode 4, for which 500 mA was used. The cell voltages varied between 3 and 15 V, the higher values being found for electrolytes of low ionic strength. Heavy water was periodically added to the cells (a few cm^3 per day) to replace the heavy water lost through electrolysis. The H:D ratio in LiOD solutions was found by NMR techniques to be $\sim 2.5\%$ after one week of running time. One sample (9) of deuterided Ti alloy powder was used for comparison with the electrochemically charged cells. This powder sample was deuterided at room temperature rather than at low temperature, in contrast to the experiments of De Ninno *et al.*¹².

Results

Figure 5a shows background data measured over 9.7 h (run 2) with no cells in the shielded housing. This figure shows only the final gated and vetoed data, as discussed above. Data taken with only the solution of LiI salt (run 4) are shown in Fig. 5c. Background data with an uncharged cell in place and with electrode 4 in solution 2 (run 5) (not shown in Fig. 5) yield no statistically significant difference in the neutron rate. In Fig. 5e we show the results obtained by using one cell containing solution 2 with higher-purity heavy water (99.8%) in conjunction with the pre-treated electrode 4 (run 6). In Fig. 5g we show the data (run 3) obtained from cells and solutions similar to those used by Jones *et al.*¹ (see Tables 1 and 2). In Fig. 6 we show neutron count rates with background subtracted for all runs 1–14, spanning almost three weeks of data collection. Cells were charged for a few days before run 1 (see Table 1), and the neutron rates at that time were typically 1 ± 1.5 counts h^{-1} above background (not shown in Fig. 6). In several runs, we used solutions and electrodes with surface and chemical treatments similar to those used by Jones *et al.* Although in some respects

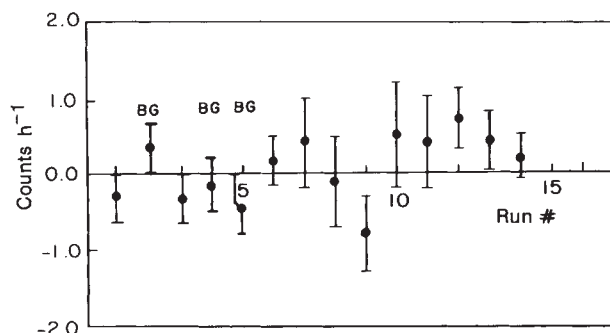


FIG. 6 Neutron count rates above average background rate (1.4 counts h^{-1}). The average rate is 0.1 ± 0.2 counts h^{-1} above background, which is significantly smaller than Jones *et al.*¹, who had the same measuring efficiency of 1%. BG indicates background runs.

our experimental arrangement may differ slightly from that of Jones *et al.* (for example, we used a platinum rather than gold electrode), the relevant features are similar. No statistically significant effect above background was observed. The average neutron count rate above background for run 6 is 0.1 ± 0.2 counts h^{-1} , a factor of 50–100 below the 15 counts h^{-1} reported by Jones *et al.*, who had a similar efficiency of 1%.

The vetoed neutron rates (at 2.5 MeV) are $\sim 35\%$ of the total detected neutron rate (Fig. 5). With the measured veto efficiency of 65% (Fig. 3), it is possible that the major portion of our neutron events (at least 50%) are induced by cosmic rays. However, as can be seen in Fig. 5, the same vetoed-event rates were observed with and without cells in the detection system, and hence it is not possible to attribute these events solely to the interaction of cosmic rays in the electrodes. We note only that cosmic rays yield neutrons of energies 1–3 MeV at a rate of ~ 1 –2 counts h^{-1} . Our sensitivity would suggest that less than 10% of these neutrons are produced by interactions of cosmic rays in the cell alone. This rate (1–2 counts h^{-1}) is only slightly below the average rate (above background) of 2 counts h^{-1} reported by Jones *et al.*, measured at a higher altitude in Utah. In addition, the presence of a peak in the spectrum of cosmic neutrons with energies of a few MeV is well-known¹³. Although time variations of cosmic-ray distributions are small, it is still possible that variations in the effect of cosmic rays in the experimental arrangement of Jones *et al.*, or barometric changes¹⁴, could account for the small effect that they reported.

These data can be used to set upper limits on d+d fusion. In particular, the data of run 6 (Fig. 5f) yielded 1.6 ± 0.2 counts h^{-1} . The average background rate (runs 2, 4 and 5) was 1.4 ± 0.2 counts h^{-1} . This yields an upper limit on the count rate resulting from the cells of 0.2 ± 0.3 counts h^{-1} above background, or a 3σ limit of 1.1 counts h^{-1} . The cell in run 6 (cell 4) includes electrode 4, consisting of 10.2 g Pd. With the assumption that the d: Pd ratio in the deuterided electrode is unity¹⁵, our neutron efficiency (1%) yields an upper limit for the cold fusion of d+d in deuterided Pd metals (at 98% confidence) of $\lambda_f < 1 \times 10^{-24}$ fusions per atom pair per second. Run 3 includes a cell similar to that used by Jones *et al.*, with two Ti electrodes of mass 2.4 g (7 and 8; see Table 1). We obtain a rate of 1.0 ± 0.2 counts h^{-1} (-0.4 ± 0.4 counts h^{-1} above background) for this run. With the assumption that the titanium has been converted to the γ -TiD₂ structure with the ratio d: Ti = 2, this yields an upper limit on the d+d fusion in charged Ti (at 98% confidence) of $\lambda_f < 6 \times 10^{-25}$ fusions per atom pair per second. Summing runs 1, 3, 6 and 7 (spanning 71.2 h of counting), with a neutron rate of 1.3 ± 0.1 counts h^{-1} (-0.1 ± 0.2 counts h^{-1} above background), we arrive at an upper limit for d+d cold fusion in deuterided Pd and Ti (at 98% confidence) of

$$\lambda_f < 2 \times 10^{-25} \text{ fusions per atom pair per second} \quad (5)$$

This rate is nearly two orders of magnitude below that suggested by Jones *et al.* ($\lambda_f \approx 10^{-23}$ fusions per deuteron pair per second). Similar limits were obtained in gases¹⁶ for molecular HD

($< 10^{-24}$ fusions per molecule per second) and for molecule D₂ ($< 10^{-26}$ fusions per molecule per second).

The γ -ray data shown in Fig. 3, from run 6 extending over 28.6 h, with the total full-energy peak efficiency at 23.8 MeV of 0.02%, yield an upper limit on the emission of γ -rays from the fusion of d+d of $\lambda_f < 2 \times 10^{-20}$ fusions per atom pair per second; here we assume that reaction channel (3) has a branching ratio of 10^{-4} . The background at the range of interest (23.8 MeV) is ~ 1 count h^{-1} per channel and the energy resolution (full width at half maximum) at this region corresponds to 6 channels. The γ -ray rate at 5.5 MeV was measured in run 13 (at which time the residual activity in the detector from ²⁴Na, produced by successive neutron calibration, was small). With the cells that contain 2.5% normal water and with the separation factor of 13 (ref. 6) this leads to a ratio D: H = 3; here we have assumed that deuterium and hydrogen are distributed uniformly throughout the lattice. With the above assumptions and using the upper limit on the γ -ray count-rate above background (see Fig. 3), we arrive at the upper limit for the fusion of p+d (at 98% confidence) of

$$\lambda_f < 1 \times 10^{-22} \text{ fusions per atom pair per second} \quad (6)$$

For a smaller separation factor of 4, the upper limit will be increased by a factor of 3.2. The p+d reaction rate of molecular hydrogen isotopes was calculated to be 10^8 times faster than the d+d reaction rate (refs 4, 5); in metals it is only 10^2 times faster (ref. 4 and S. E. Koonin, personal communication). Together with the d+d fusion rate suggested by Jones *et al.*¹, we would expect the p+d rate to be $\sim 10^{-21}$ fusions per atom pair per second, a factor of 10 greater than our measured upper limit.

Conclusions

We have carried out a number of tests of some of the claims regarding cold fusion made by Fleischmann *et al.*² and Jones *et al.*¹. We have attempted to reproduce the electrochemical conditions used by them, and have also used a sample deuterided under high-pressure gas. We have searched for the neutrons and γ -rays that must be emitted from the putative cold-fusion processes. Our experiments, with high sensitivity and small background, have revealed no statistically significant deviation from background. The upper limits that we deduce for the rates of cold fusion of d+d and p+d in metals are considerably smaller than those reported in refs 1 and 2. The average neutron detection rates above background reported by Jones *et al.*¹ (measured with the same efficiency as here) are consistent with the rate that we measure for neutrons originating from cosmic rays. It is therefore likely that a significant fraction of the neutrons measured by Jones *et al.* may be attributed to cosmic rays. The absence of neutrons and γ -rays emitted from these cold-fusion systems, together with the absence of a known mechanism for the suppression of reactions (1), (3) and (4), raises serious doubts as to whether cold fusion does occur in deuterided Pd and Ti metals at the rates suggested by Fleischmann *et al.*² and Jones *et al.*¹. $\square$

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Expression of a large family of POU-domain regulatory genes in mammalian brain development

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A novel region referred to as the POU-domain is present in two tissue-specific transcription factors, Pit-1 and Oct-2, that activate expression of genes specifying pituitary and lymphocyte phenotypes. We report the identification of multiple new members of a large family of POU-domain genes expressed in adult brain, and document that all the known mammalian POU-domain genes, including *Pit-1* and *Oct-2*, are expressed widely in the developing nervous system.

BRAIN development involves an intricate programme of gene expression that leads to the establishment of many neuronal phenotypes and a precise, complex pattern of connections between them. Although multifactorial interactions among neuronal surface proteins have been proposed to mediate growth-cone guidance and neuronal recognition during the establishment of this morphological network (reviewed in refs 1, 2), the nuclear events underlying neuronal development and the accompanying diverse patterns of specific gene expression remain largely unknown. It is likely, however, that the temporal and spatial patterns characteristic of mammalian brain development reflect sequential activation of a complex array of regulatory factors similar to those presumed to account for establishing structural patterns in *Drosophila*³⁻⁹. Morphogenesis in *Drosophila* is regulated, in part, by a family of genes that contains a 180 base-pair (bp) homoeobox DNA sequence encoding a conserved 60-amino-acid sequence referred to as the homoeodomain. It is widely accepted that these homoeodomain proteins bind to specific DNA elements and exert their effects through transcriptional regulation¹⁰⁻¹⁴. Genes containing sequences homologous to the homoeobox have since been isolated from a number of vertebrates¹⁵, including mammals^{16,17}, implying their potential roles in the development of these higher organisms.

Recently, three mammalian transcription regulators, Pit-1, Oct-2 and Oct-1, have been characterized and shown to contain a region with distinct homology to the homoeodomain of *Drosophila* regulatory gene products¹⁸⁻²⁴. In the adult, Pit-1 is expressed exclusively in the pituitary and activates the transcription of the prolactin and growth hormone genes^{18,25}, whereas Oct-2 is expressed in lymphoid B cells and activates the transcription of immunoglobulin genes²¹⁻²³. Oct-1 is believed to be ubiquitously expressed²⁴. Thus, Pit-1 and Oct-2 are involved in at least certain stages of the development of cellular phenotypes in the mammalian endocrine and immune systems, respectively. Comparison of Pit-1, Oct-2 and Oct-1 with a *Caenorhabditis elegans* regulatory gene product, unc-86 (ref. 26), a genetically-defined determinant of cell-specific developmental fate²⁷, revealed that the four proteins share two homologous regions

(Fig. 1a): a 60-amino-acid homoeodomain divergent from the *Drosophila* homoeodomain, called the POU-homoeodomain, and a 76-78-amino-acid region that is unique to these four proteins, referred to as the POU-specific domain^{28,29}. These two regions, together with a non-conserved region between them, constitute the POU-domain (Fig. 1a).

We now report the identification of four new mammalian POU-domain genes, referred to as *Brain-1* (*Brn-1*), *Brain-2* (*Brn-2*), *Brain-3* (*Brn-3*) and *Testes-1* (*Tst-1*), that are expressed in the nervous system and, in some cases, a limited number of other organs. These new genes, as well as those encoding Pit-1, Oct-1 and Oct-2, are widely expressed in the developing neural tube, and all (but the Pit-1 encoding gene) exhibit differential, overlapping patterns of restricted expression in the adult brain. *Pit-1* provides an example of a biphasic pattern of expression and is not present in the mature brain.

Identification of new genes encoding POU-domain proteins. A strategy was devised to isolate new members of the POU-domain gene family. Degenerate oligonucleotides representing all possible codons for two 9-amino-acid residues conserved among Pit-1, Oct-1, Oct-2 and unc-86 were used as primers for the polymerase chain reaction (PCR)³⁰. DNA complementary to poly(A)-selected RNA from human brain, and rat brain and testes were used as templates. Four new members of the POU-domain gene family were identified. Three clones were isolated from brain complementary DNA: *Brn-1* and *Brn-2* from human, and *Brn-3* from rat; *Tst-1* was isolated from rat testes cDNA. Predicted amino-acid sequences for each new member are shown in Fig. 1b.

Structural comparisons of the four new POU-domain proteins revealed that they are highly related to Pit-1, Oct-1, Oct-2 and unc-86. Three of the new proteins, Brn-1, Brn-2 and Tst-1, are highly homologous, with 94% of the amino-acid residues being identical among them throughout the entire POU domain (Fig. 1b), whereas DNA sequence similarity between any two is 81-89% (data not shown). Brn-3 is highly related to unc-86, sharing 79% amino-acid identity, including three characteristic additional amino-acid residues in the region between the A and B portions of the POU-specific domain (Fig. 1b). Oct-1 and Oct-2, as noted previously^{22,24}, resemble each other across the entire POU domain (80% identity). Pit-1 is distinct from the other seven proteins in the POU-domain family at several amino-acid positions that otherwise would be fully conserved, and is thus far the most divergent member of the family. Based on these similarities, the eight known POU-domain proteins appear to constitute a family that segregates into four classes, arbitrarily referred to as POU-I (Pit-1), POU-II (Oct-1, Oct-2), POU-III (Brn-1, Brn-2, Tst-1), and POU-IV (Brn-3, unc-86). The N-terminal basic part of the POU-homoeodomain is identical for the first 11 or 12 amino acids in each class, including the initial cluster of basic residues. In addition, 15-18 amino acids in the C-terminal 'WFC' region (Fig. 1a) are particularly well-conserved between all members of each class. A consensus

sequence for the POU domain is shown in Fig. 1b, emphasizing the high degree of primary amino-acid sequence conservation among all eight family members. Comparisons between classes are presented in Fig. 1c, showing that amino-acid sequences are most conserved in the POU-specific domain.

Spatially and temporally restricted patterns of expression of POU-domain gene family. *Brn-1*, *Brn-2*, *Brn-3* and *Tst-1* were all expressed in the brain (Fig. 2). *Brn-2* cDNA hybridized to transcripts of different lengths in the hypothalamus (6.1 kilobases (kb)) and hippocampus (7.7 kb) (Fig. 2b), whereas the hybridization signal for *Brn-1* transcripts was less intense, and >7 kb in size (data not shown). *Brn-3* cDNA hybridized to a 7.8-kb RNA species in brain, and also weakly to a 7-kb transcript in spleen (Fig. 2b). *Tst-1*-reactive species included a 4.4-kb poly(A)⁺ RNA in testes, and a 7.6-kb brain transcript (Fig. 2b). RNase protection assays confirmed the identity of the reactive RNAs as authentic transcripts of each gene (Fig. 2a).

Hybridization histochemistry was used to determine whether these transcripts exhibited either widespread or restricted patterns of expression in the central nervous system (CNS). Representative examples of this analysis are shown in Fig. 3 and a summary of the complete survey is presented in Table 1. *Brn-1* and *Brn-2* exhibited virtually identical patterns of expression in the CNS, although *Brn-1* was clearly expressed in the medullary zone of the kidney, whereas *Brn-2* was not. Hybridization for *Brn-1* and *Brn-2* was found in at least some classes of neurons at all levels of the neuraxis. They are the most widely expressed members of the POU-domain family examined in the brain (Table 1). Notably, almost all regions of the cerebral (layers II–V) and cerebellar (Purkinje cells) cortices specifically hybridized as did the basal forebrain cholinergic system, ventral midbrain dopamine system, paraventricular and supraoptic neuroendocrine system, somatic motoneurons in the cranial nerve nuclei and ventral horn, and tectum.

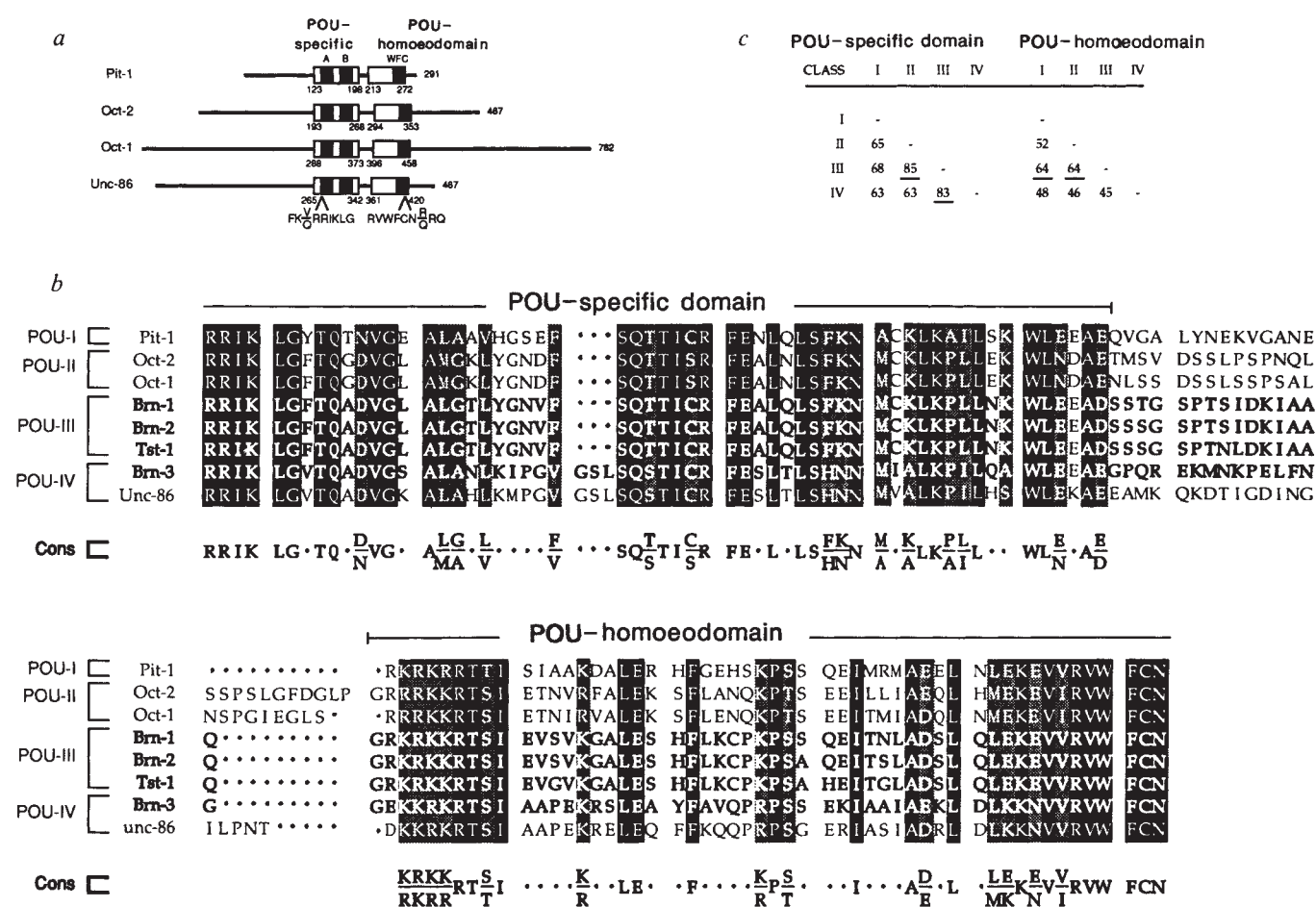


FIG. 1 Amino-acid sequences of the POU-domains of four new mammalian POU-domain genes. **a**, Schematic representation of four previously characterized POU-domain proteins, referred to as Pit-1 (refs 18, 19), Oct-1 (ref. 24), Oct-2 (refs 21–23), and *unc-86* (ref. 26), with the locations of the POU-domains indicated. Two highly conserved amino-acid regions upon which degenerate oligonucleotides for use in the polymerase chain reaction (PCR) were designed, are indicated. **b**, Partial amino-acid sequences of four new POU-domain genes inferred from DNA sequences, and compared to the corresponding sequences of Pit-1, Oct-1, Oct-2 and *unc-86*. Based on sequence similarities, these proteins fall into four classes: POU-I (Pit-1), POU-II (Oct-1, Oct-2), POU-III (Brn-1, Brn-2, Tst-1), and POU-IV (Brn-3, *unc-86*). The black background indicates full conserved residues; gray indicates two generally conservative amino-acid residues. The POU-specific and POU-homoeodomains are overlined, and a consensus sequence for all eight proteins is presented. **c**, The similarity of POU-specific and POU-homoeodomains between classes is presented.

METHODS. DNA (50 ng) complementary to poly(A)-selected RNA prepared from human fetal brain (generating Brn-1 and Brn-2), rat brain (generating Brn-3), and rat testes (generating Tst-1) was prepared as previously described¹⁸. PCR was performed using 1.5-μg fully degenerate oligonucleotides corresponding to the sequences Phe-Lys-Val/Gln-Arg-Arg-Ile-Lys-Leu-Gly and Arg-Val-Trp-Phe-Cys-Asn-Gln/Arg-Arg-Gln and Cetus Taq polymerase³⁰; 30–40 cycles were performed at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 3 min. The reaction products were cloned into pBKS (Stratagene) and subjected to DNA sequence analysis using T7 polymerase (Sequenase) on a double-stranded DNA template⁴⁴. All sequencing was performed on two to three independent clones, in both sense and anti-sense strands. In a successful PCR and cloning process, $\frac{1}{3}$ to $\frac{2}{3}$ of the colonies were positive by sequencing or restriction diagnosis. The numbers of positive isolates did not necessarily reflect the abundance of the messenger RNA. Attempts to PCR cDNA from rat heart, liver and pancreas for POU-domain genes were unsuccessful.

TABLE 1 Distribution of POU-domain mRNA expression in the adult rat nervous system determined by *in situ* hybridization

	<i>Pit-1</i>	<i>Brn-1</i>	<i>Brn-2</i>	<i>Brn-3</i>	<i>Tst-1</i>	<i>Oct-1</i>	<i>Oct-2</i>
Sensory ganglia	—	—	—	++++	—	—	—
Cortex							
Isocortex, layers 2-5	—	+	+	—	—	—	—
layers 5-6	—	—	—	—	+	—	—
Olfactory bulb, mitral	—	+	+	—	+	—	—
periglomerular	—	+	+	—	—	—	—
Islands of Calleja	—	+++	++	—	—	—	—
Piriform	—	+	+	—	+	—	—
Presubiculum, layer 2	—	++	++	—	—	—	—
CA1/subiculum	—	++	++	—	+++	—	—
Dentate gyrus	—	+	+	—	—	—	—
Subependymal zone	—	++	++	—	—	—	—
Amygdala							
N. lateral olfactory tract	—	+	+	—	++	—	—
Septum							
Medial n./n. diagonal band	—	+	+	—	—	—	—
Bed n. stria terminalis	—	+	+	—	—	—	—
Basal ganglia							
Striatum	—	—	—	—	+	—	—
Substantia innominata	—	+	+	—	—	—	—
S. nigra/ventral tegmental a.	—	+	++	—	+	—	—
Thalamus							
Medial habenula	—	+++	++	+++	++	++	—
Lateral habenula	—	—	—	++	—	—	—
Parafascicular n.	—	+	+	—	—	—	—
Reticular n.	—	+	+	—	—	—	—
Zona incerta	—	+	+	—	—	—	—
Hypothalamus							
Suprachiasmatic n.	—	—	—	—	—	—	++
Supraoptic/paraventricular n.	—	++	+++	—	—	—	—
Medial preoptic n.	—	+	+	—	—	—	—
Tuberomammillary n.	—	+	+	—	—	—	—
Lateral mammillary n.	—	+	+	—	—	—	—
Medial mammillary n.	—	—	—	—	—	+	++
Posterior hypothalamic a.	—	+	+	++	—	—	—
Brainstem-sensory							
Superior colliculus, inter, gray	—	+	+	+	+	—	—
Parabigeminal n.	—	++	+++	—	+++	—	—
Superior olive	—	—	—	+	—	—	—
Inferior colliculus, dorsal n.	—	+	+	++	—	—	—
Area postrema	—	+	++	—	—	+	—
Brainstem-motor							
Motor n. trigeminal	—	+	+	—	—	—	—
Facial n.	—	+	+	—	—	—	—
Hypoglossal n.	—	+	+	—	—	—	—
N. ambiguus	—	—	—	++	—	—	—
Dorsal motor n. vagus	—	—	—	—	++	—	—
Brainstem-core							
Periaqueductal gray	—	+	+	+	—	—	—
N. incertus	—	++	++	—	—	—	—
Interpeduncular n.	—	—	—	+	—	—	—
Cerebellum and related							
Deep nuclei	—	+	+	—	—	—	—
Purkinje	—	++	++	—	—	—	—
Granule	—	—	—	—	++	+++	++
Red n.	—	—	—	—	—	—	+
Inferior olive	—	+	+	++++	—	—	—
Spinal cord							
Ventral horn	—	+	+	—	—	—	—

Relative density of *in situ* hybridization indicated as follows: —, not detected; +, low; ++, moderate; +++, high; +++++, very high. Sense-strand control hybridization was (—) for all regions indicated.

In contrast, the *Brn-3* gene transcript exhibited a more restricted pattern, with dense hybridization limited to the habenula, posterior hypothalamic area, inferior olive, inferior colliculus, and nucleus ambiguus. Furthermore, it was the only clone examined that hybridized to sensory ganglion cells (in the trigeminal and dorsal root ganglia, which are derived from the neural crest, and in the spiral ganglion). The *Tst-1* gene was expressed in the cerebral (layers V–VI) and cerebellar (granule cells) cortices as well as in the medial habenula, superior colliculus and parabigeminal nucleus, and dorsal motor nucleus

of the vagus. Thus, each gene exhibited a unique, restricted pattern of expression.

The developmental patterns of expression of the four new POU-domain genes were examined from rat embryonic day 8 through birth (see Table 2 and Fig. 4 for examples). All four were widely expressed in all levels of the neural tube (including the retina) during at least some part of this period. Hybridization in the ventricular (proliferative) zone of the neuroepithelium, which gives rise to neurons in CNS, was also evident for all four probes. As shown in Table 2, however, the intensity of

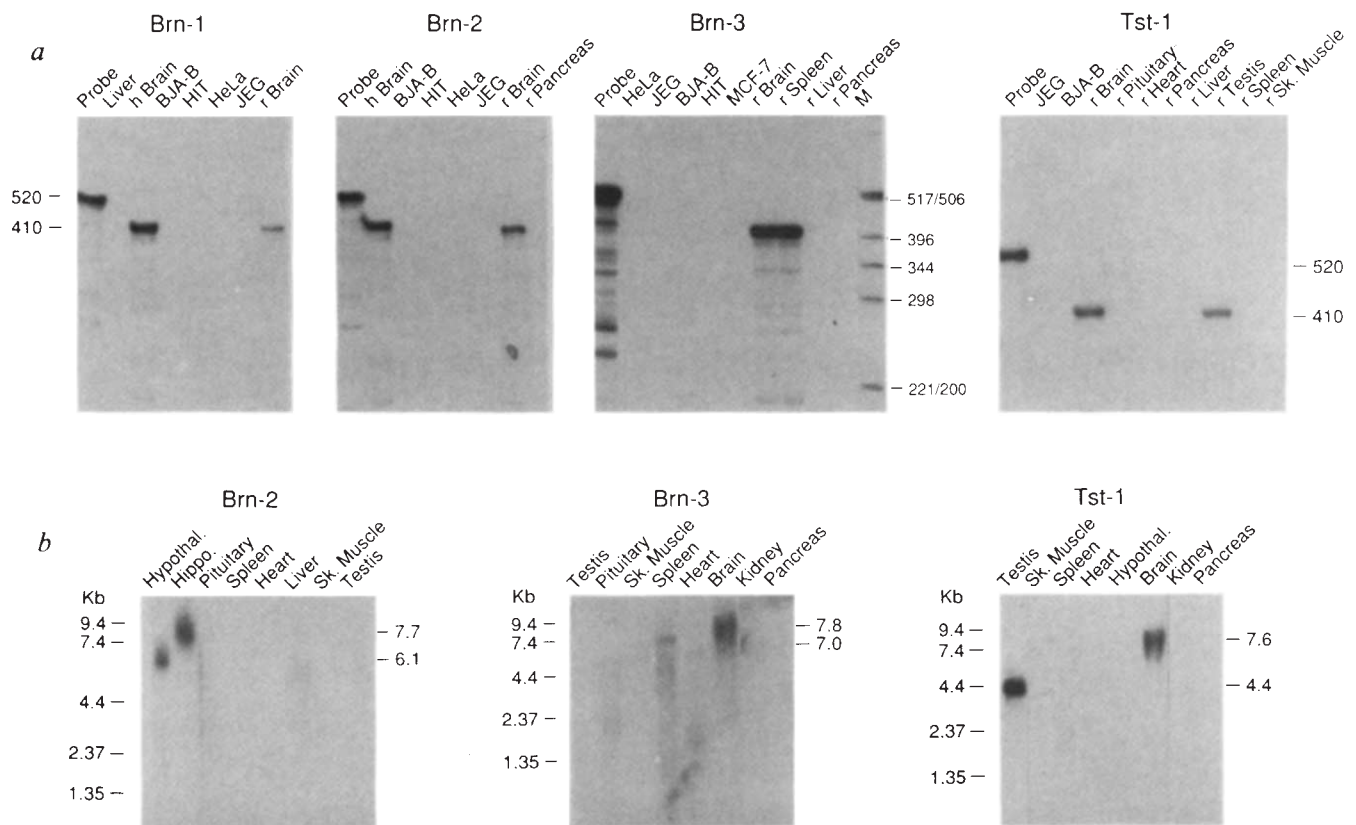


FIG. 2 RNase protection and northern analysis of *Brn-1*, *Brn-2*, *Brn-3* and *Tst-1* transcripts. **a**, Autoradiograph of RNase protection assays of *Brn-1*, *Brn-2*, *Brn-3* and *Tst-1* in a limited number of rat (r) and human (h) tissues and BJA-B (human lymphoid B-cell line), HIT (hamster pancreas island cell line), JEG (human placental cell line) and MCF-7 (human breast cell line). For each analysis the size of the full probes (520–529 nucleotides) and protected probes (410–419 nucleotides) are indicated. Probes from all clones were protected in the brain, with additional protection in one peripheral organ for *Brn-3* (spleen) and *Tst-1* (testes). Protection of the full-length probes across species indicates extensive conservation at the nucleotide level, with no consecutive nucleotide substitutions, as was confirmed by comparison of human and rat *Oct-2* POU-domain (100% identity of amino-acid residues and 92% identity of nucleotide residues; data not shown). **b**, Northern blot analysis of *Brn-2*, *Brn-3* and *Tst-1* in rat tissues. Migration of the RNA standard and sizes of reactive species are noted. All clones exhibited

reactive species in various regions of the rat (r) brain, with additional species in spleen (*Brn-3*) and testes (*Tst-1*).

METHODS. RNA was isolated¹⁸ and 20 µg of total RNA was subjected to RNase protection analysis as previously described²⁴. Anti-sense RNA probes were transcribed using T3 RNA polymerase in the presence of ³²P-UTP, generating RNA run-offs of 520 nucleotides (*Brn-1*, *Brn-2*, *Tst-1*) and 529 nucleotides (*Brn-3*). Mature transcripts protected 410 nucleotides of probe for *Brn-1*, *Brn-2*, *Tst-1* and 419 nucleotides of probe for *Brn-3*, respectively. RNA blot analysis was performed with 5 µg of poly(A)-selected RNA from rat tissues. RNAs in each lane were intact based on staining of ribosomal RNA and hybridization with other cDNA probes. RNA was denatured, separated on 1.2% agarose-formaldehyde gels, blotted, and hybridized using 10⁶ c.p.m. ml⁻¹ of labelled probe, at 42 °C for 12–16 h, as previously described¹⁸. All blots were washed in 0.2 × SSC at 65 °C. Autoradiographs were exposed for ~24 h.

TABLE 2 Distribution of POU-domain mRNA expression in the embryonic rat nervous system determined by *in situ* hybridization

	<i>Pit-1</i>	<i>Brn-1</i>	<i>Brn-2</i>	<i>Brn-3</i>	<i>Tst-1</i>	<i>Oct-1</i>	<i>Oct-2</i>
e8							
Egg cylinder	–	+	+	–	+++	++	–
e10							
Neural plate/tube	+	+++	++++	(+)	++	++	(+)
e13							
Telencephalon	+	+++	++++	–	++	+	–
Diencephalon	+	+++	++++	–	++	+	++
Brainstem	+	+++	++++	++++	++	+	++
Spinal cord	+	+++	++++	++++	++	+	++
Retina	(+)	–	–	–	+	+	+
Sensory ganglia	–	–	–	+++	–	–	+
e16							
Telencephalon	–	+++	++++	–	+	–	+
Diencephalon	–	+++	++++	–	+	+	++
Brainstem	–	+++	++++	++++	+	–	++
Spinal cord	–	+++	++++	++++	–	–	++
Retina	–	+	+	++++	+	+	+++
Sensory ganglia	–	–	–	++++	–	–	+

Relative density of *in situ* hybridization: –, not detected; (+), very low; +, low; ++, moderate; +++, high; +++++, very high. Sense-strand control hybridizations were negative for all regions indicated.

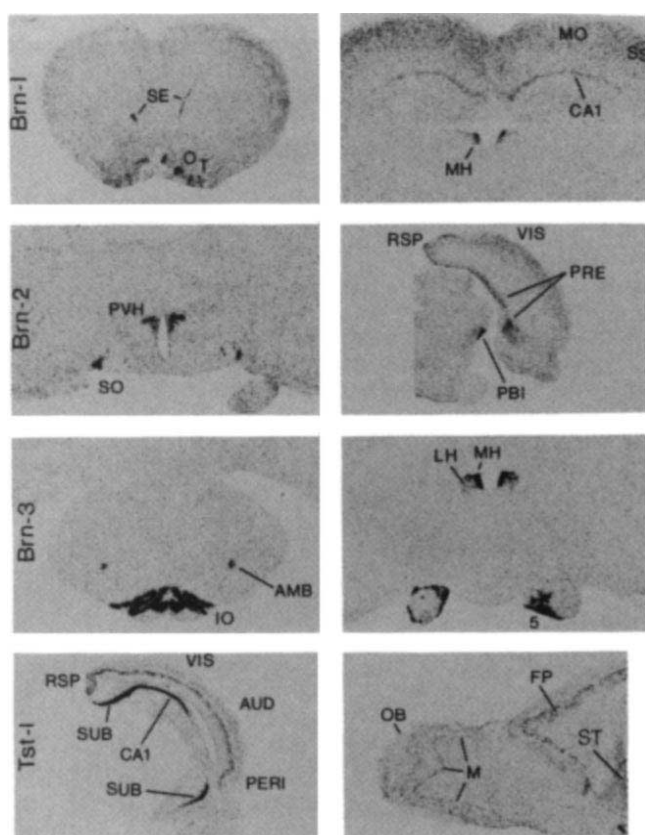


FIG. 3 Representative hybridization histochemistry of mRNA in adult rat brain using *Brn-1*, *Brn-2*, *Brn-3* and *Tst-1* anti-sense probes. *Brn-1* hybridization is shown in the subependymal zone (SE), islands of Calleja in the olfactory tubercle (OT), medial habenula (MH), pyramidal cells in field CA1 of the hippocampus (CA1), and layers II–V of the isocortex, including motor (MO) and somatosensory (SS) areas (left $\times 3$, right $\times 5$). *Brn-2* hybridization is shown in the magnocellular and dorsal medial parvocellular parts of the paraventricular (PVH) and supraoptic (SO) nuclei of the hypothalamus, in the presubiculum (PRE), retrosplenial (RSP), and visual (VIS) parts of the isocortex, and in the parabrachial nucleus (PBI) (left $\times 4.5$, right $\times 2$). *Brn-3* hybridization is shown in the inferior olive (IO), nucleus ambiguus (AMB), lateral (LH) and medial (MH) habenula, and trigeminal ganglion (5) (left $\times 4.5$, right $\times 4$). *Tst-1* hybridization is shown in the retrosplenial (RSP), visual (VIS), auditory (AUD) and perirhinal (PERI) areas of the cerebral cortex, as well as in the subiculum (SUB) and field CA1 (CA1) of the hippocampus, mitral cells (M) in the olfactory bulb (OB), layers 5 and 6 in the frontal pole (FP) of the isocortex and in the striatum (ST) (left $\times 2$; right $\times 4$, sagittal).

METHODS. As described in detail previously^{45,46}. Briefly, adult male Sprague–Dawley rats were perfused with 4% paraformaldehyde. Brains were removed and post-fixed overnight before freezing and preparation of serial, generally coronal sets of 20- μ m thick sections throughout the brain. Representative sets (one in three) were stained for cytoarchitectonic analysis. Others were attached to poly-L-lysine-coated slides for proteinase K treatment (0.01 mg ml⁻¹, 30 min at 37 °C). Sections were hybridized for 18 h at 59 °C with ³⁵S-labelled single-stranded RNA probes transcribed from vectors containing the specific clones described above. Slides were treated with RNase A (20 μ g ml⁻¹), washed 30 min in 0.1 $\times$ SSC at 60–65 °C, dehydrated, and exposed to Amersham Hyperfilm beta max for 24–72 h. For microscopic evaluation of hybridization over single cells, the slides were defatted and dipped in Kodak NTB2 autoradiographic emulsion (diluted 1:1 with distilled water), dried in a humid chamber (2–4 h), exposed desiccated in the dark at 4 °C for 2 weeks, and developed with Kodak D-19, followed by Rapid Fixer.

hybridization signals, and the time course of this anatomical restriction in the developing neural tube, for *Brn-1* and *Brn-2* together, for *Brn-3*, and for *Tst-1* were distinct, and the patterns tended to reflect the adult loci of expression. Clear hybridization was also observed in the mantle layer, in the early cortical plate (embryonic day 18 for *Brn-1*, *Brn-2*, *Tst-1*; see Fig. 4c), and in the external granular layer of the cerebellum (*Tst-1*). By the day of birth (PO) *Brn-2* and *Tst-1* are still present in the proliferating cells of the subependymal zone, but now exhibit a clear difference in the laminar pattern of expression in the cortical plate. In addition to expression in the superficial part of the cortical plate, *Brn-2* can be seen in bands of young neurons (see MSN, Fig. 4c) that are presumably migrating through the cortical plate eventually to form the superficial layers of the adult cortex, where *Brn-2* is normally expressed (isocortical layers II–V). The cells in the superficial part of the cortical plate on the day of birth express *Tst-1*; these cells eventually constitute the deep layers of the cortex (layers V, VI) where *Tst-1* is expressed in the adult. These data indicate that the genes are differentially expressed during the migratory phase of at least some types of young neurons. RNase protection analyses confirmed the temporal expression pattern of these cloned genes (Fig. 4a).

Neuronal expression of *Pit-1*, *Oct-1* and *Oct-2* transcripts. In contrast to the four new POU-domain family members that are expressed in the neural tube during development and in the mature brain, *Pit-1* and *Oct-2* have been thought to be expressed exclusively in non-neuronal tissues, the anterior pituitary and lymphoid B cells, respectively. Unexpectedly, *Pit-1* transcripts were detected in the neural plate and tube on embryonic days 10–13 and disappeared thereafter as assessed by *in situ* hybridization and RNase protection assays (Fig. 5a, b, Table 2). On embryonic day 16, *Pit-1* transcripts reappeared and were expressed exclusively in the developing anterior lobe of the pituitary gland and thereafter could not be detected in any brain region (Fig. 5b). Thus, *Pit-1* exhibits a biphasic pattern of

expression: it is initially expressed widely in the neural tube, is subsequently restricted completely from the nervous system, and later reappears in a distinct tissue, the anterior pituitary gland. The biphasic pattern of *Pit-1* expression is reminiscent of *Drosophila* homoeodomain genes such as *fushi tarazu* (*ftz*), which is expressed early in development to play a critical role in pattern formation, and later reappears in a limited number of neurons³¹.

An unexpected result for the expression patterns of *Oct-2* was also observed. The *Oct-2* genes were widely expressed in the developing neural tube including the diencephalon, brainstem, spinal cord, and sensory ganglia (Fig. 5a, c and Table 2). But, unlike *Pit-1*, *Oct-2* transcripts were found in the brain of the adult animal, with expression limited to the medial mammillary nucleus, suprachiasmatic nucleus (Fig. 5c), which is involved in generating circadian rhythms, cerebellar granule cells, and red nucleus (Table 1). RNase protection assays confirmed expression of the *Oct-2* gene in the brain (Fig. 5a).

It has been suggested that *Oct-1* is ubiquitously expressed²⁴. Thus, based on the expression of *Pit-1* and *Oct-2*, it was of interest to determine whether *Oct-1* was also expressed during neurogenesis. The *Oct-1* gene was indeed found to be widely expressed in the neural tube (embryonic day 13), but at much lower levels than any of the other POU-domain genes (Fig. 5d and Table 2). In adult brain, *Oct-1* expression was highly restricted to cerebellar granule cells (Fig. 5d) and to the medial habenula, medial mammillary nucleus, and area postrema (Table 1). *Oct-1* transcripts were also detected in thymus, lymph nodes (Fig. 5d), spleen, thyroid, ovary, testes and mammary tissue, but not in kidney, heart, liver or salivary gland (data not shown). This is a much more widespread pattern of expression than was observed for any other member of the family.

The potential early expression of the POU-domain gene family before induction of the neural plate was examined on embryonic day 8 (Table 2). *Oct-1* (Fig. 5d), and *Tst-1* were highly expressed in the egg cylinder, whereas weaker hybridization signals were

detected for *Brn-1* and *Brn-2* (Table 2). In contrast, *Pit-1*, *Oct-2* and *Brn-3* transcripts were not detected at this stage.

When the adult pattern of neuronal expression of the six mammalian POU-domain genes (all but *Pit-1*) are compared (Table 1), several intriguing features emerge. First, none of the patterns for individual members of the family corresponds entirely to any known topographical division or functional system, to the distribution of any known neurotransmitter or receptor, or to any one cell type. Second, some regions of the nervous system appear to express only one of the members examined here, whereas other regions clearly express multiple members of the family. In some instances different cell types in a particular region express different members of the family. The clearest example of this is in the cerebellum, where *Brn-1* and *Brn-2* are expressed in Purkinje cells, whereas *Tst-1*, *Oct-1* and *Oct-2* are expressed in granule cells. Only one of the known POU-domain proteins, *Oct-2*, is expressed in the suprachiasmatic nucleus, whereas *Brn-1*, *Brn-2*, *Brn-3*, *Tst-1* and *Oct-1* are all expressed in the medial habenula. It would therefore appear that neurons in a region like the medial habenula may express as many as five of the seven mammalian POU-domain genes identified thus far, whereas there are many brain regions where no expression of any known POU-domain protein is detected.

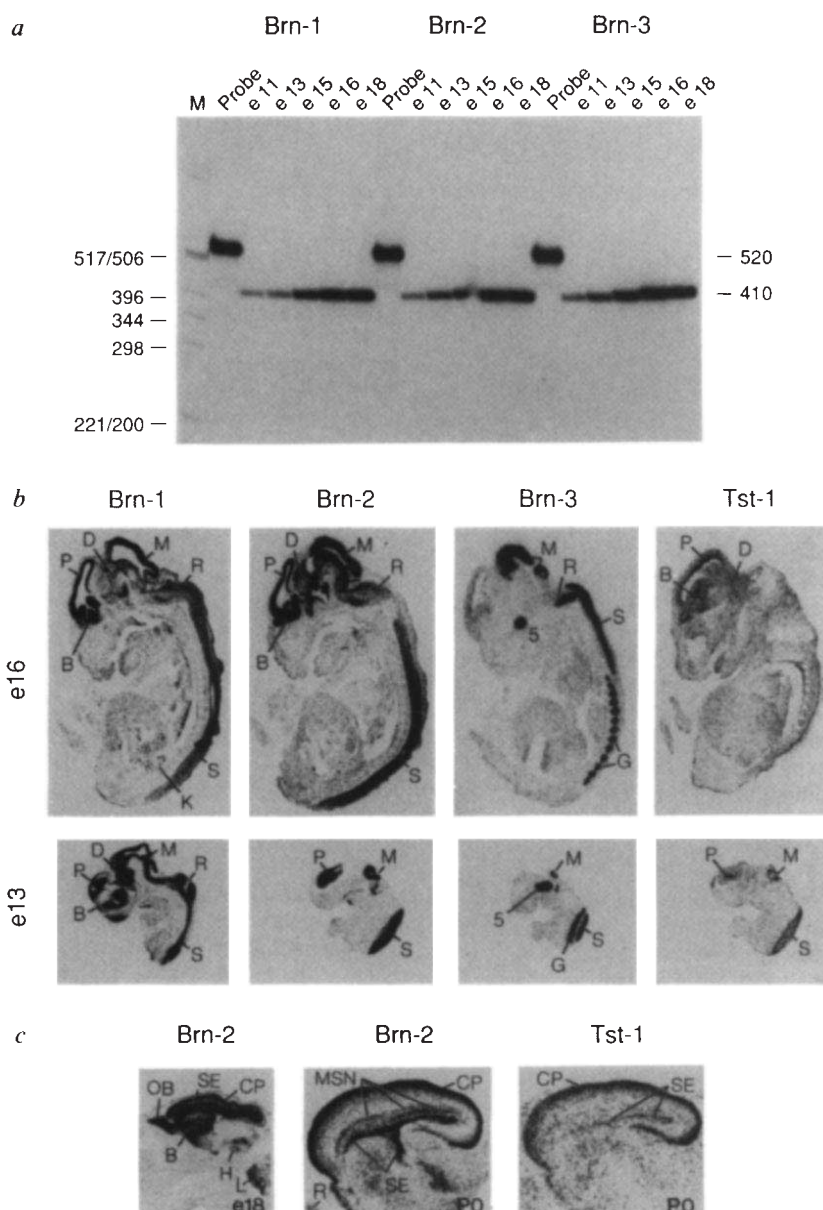
Discussion

The identification of multiple new POU-domain genes, and their extensive homology in the POU-domain with previously known genes, firmly strengthen the notion of a large family of the POU-domain proteins. In fact, POU-domain genes have also been found to express in *Drosophila* and share up to 94% amino-acid identity in the POU-domain with the mammalian genes (M.N.T. and X.H., unpublished results). Based on preliminary data it is likely that the mammalian POU-domain gene family is much larger than the seven identified members.

All the known mammalian POU-domain genes exhibit distinct temporal and spatial patterns of expression during brain development, with wide expression in the neural tube, and different patterns of subsequent restriction, suggesting that they may play a role in the development of neuronal phenotypes. This is demonstrated by the analysis of *Brn-2* and *Tst-1* gene expression during cerebral cortical development, which involves the progressive generation of neurons from a proliferative zone and their sequential migration to the more superficial layers in which they ultimately reside^{32,33}. Initially, both *Brn-2* and *Tst-1* are widely expressed in the proliferative zone and early cortical plate; later, although still present in the proliferative zone, their

FIG. 4 Embryonic patterns of expression of *Brn-1*, *Brn-2*, *Brn-3* and *Tst-1*. **a**, RNase protection analysis of *Brn-1*, *Brn-2* and *Brn-3* during rat embryonic development. Total RNA (20 µg) was prepared¹⁸ from embryonic days 11–18 (e11, e13, e15, e16, e18) and hybridized to anti-sense ³²P-labelled RNA probes. Size of probes and protected species are given in Fig. 2. **b**, Parasagittal sections through an embryonic day 13 (e13) and 16 (e16) rat were hybridized with ³⁵S-labelled anti-sense RNA probes and representative examples of resulting autoradiographs are shown. Hybridizing regions include: brainstem (B), diencephalon (D), dorsal root ganglia (G), mesencephalon (M), pallium (P), rhombencephalon (R), spinal cord (S), and trigeminal ganglion (5). Notice that *Brn-1* also hybridizes to discrete tubular structures in fetal kidney (K). No hybridization was observed using sense-strand probes. **c**, *Brn-2* and *Tst-1* expression during the migratory phase of cortical neurogenesis. Hybridization of *Brn-2* on e18 and day of birth (PO) showing expression at an early phase of cortical plate formation and at a stage (PO) when migrations that underlie the mature pattern of lamination are becoming evident. Hybridization transcripts are seen in the olfactory bulb (OB), subependymal zone (SE), cortical plate (CP), basal ganglia (B), hypothalamus (H), and membranous labyrinth (L) of the inner ear. On day PO waves of migratory neurons destined for the superficial layers (migratory superficial cortical neurons, MSN), are observed, along with hybridization in the retina (R). Hybridization of *Tst-1* to an adjacent section reveals labelled cells in the cortical plate that will later, form the deep cortical layers.

METHODS. All techniques used were described in the legend to Figs 2 and 3 except that sections were pretreated with 0.5% Triton-X100 instead of proteinase K. For fetal material, the morning that the vaginal plug was detected is defined as embryonic day 0 (e0).



laminar expression is restricted to different layers that reflect the mature laminar pattern (*Brn-2* in layers II–V and *Tst-1* in layers V–VI). Moreover, *Brn-2* transcripts can be seen in bands of neurons that presumably represent the migrating neurons from the proliferative zone towards the superficial layers. Therefore, the expression of *Brn-2* and *Tst-1* is correlated with all stages in the establishment of cortical lamination. In *C. elegans* a POU-domain gene, *unc-86*, determines the development of neuronal phenotypes involving sensory neurons^{26,27}. One of the new POU-domain genes, *Brn-3*, is highly homologous to *unc-86*, and is expressed in sensory ganglion cells. The similarity between the protein sequence and the patterns of expression in such diverged organisms as nematodes and mammals suggests highly conserved roles for the two proteins.

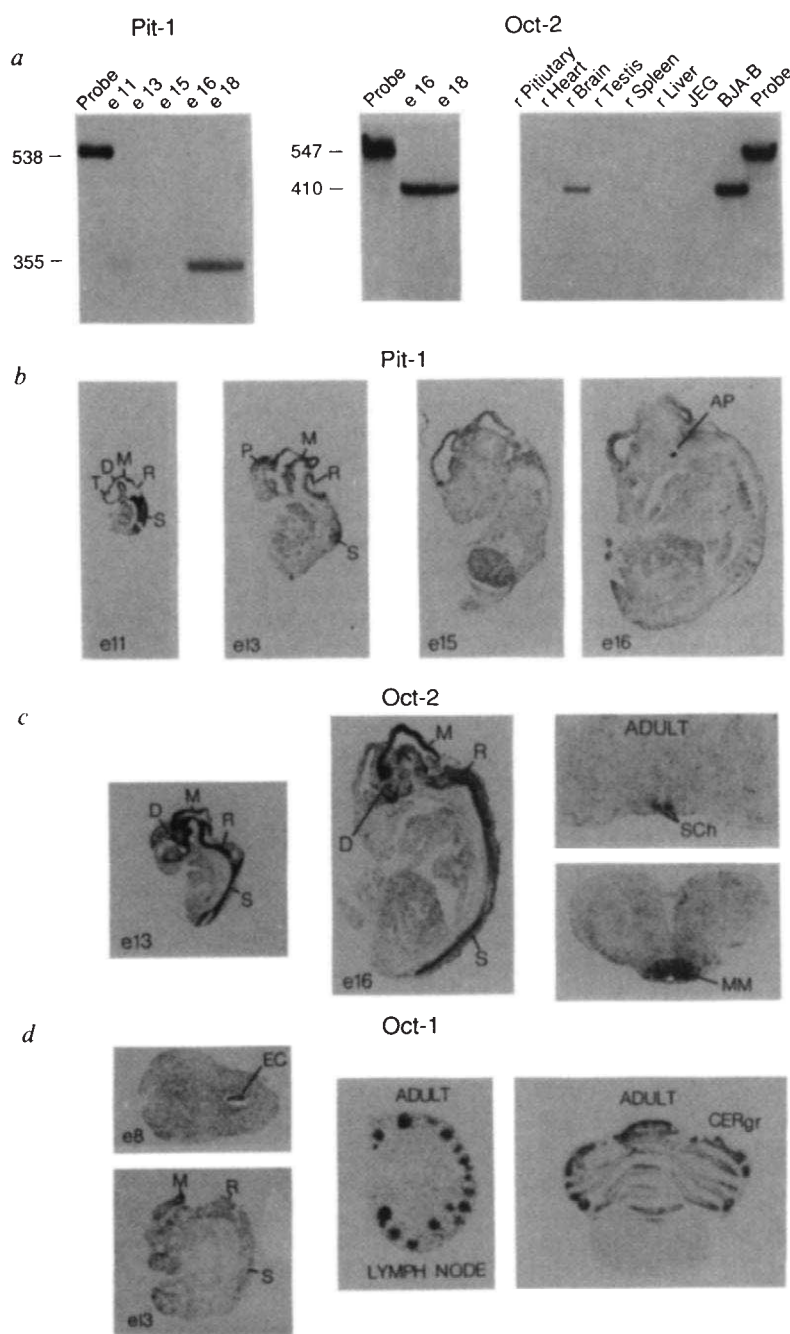
Transient expression of *Pit-1* in neural tube and continuous expression of *Oct-2* in neural tube and adult brain imply their role in neurogenesis, in addition to their well characterized transcription activation function in mature pituitary (*Pit-1*) and B lymphocytes (*Oct-2*). By analogy, it seems likely that all

members of the POU-domain gene family exert their effects by modulating specific patterns of gene transcription.

Although each member of the POU-domain gene family has a distinct expression pattern, their co-expression in some brain regions is apparent. At the same time, some mammalian POU-domain proteins co-exist with certain classical homoeodomain proteins^{16,34–39}. Genetic studies have shown that, in addition to their classical role in pattern formation, homoeodomain proteins also participate in neuronal development, as in the case of the products of the *ftz*³¹, *cut*⁴⁰ and *rough*⁴¹ genes in *Drosophila*, and the *mec-3* (ref. 42) gene in *C. elegans*. In fact, the products of *unc-86* and *mec-3* act within a regulatory hierarchy that specifies the same differentiation pathway for the same set of sensory neurons⁴³. Thus, it is conceivable that co-expression of POU-domain proteins, in concert with other homoeodomain proteins, contributes to a combinatorial code critical for mature cellular phenotypes in the mammalian nervous system, as well as in other organ systems. □

FIG. 5 Embryonic and adult patterns of *Pit-1*, *Oct-2* and *Oct-1* gene expression. *a*, Autoradiographs of RNase protection hybridization of *Pit-1* (e11–e18) and *Oct-2* (e16–e18) transcripts in embryonic and mature rat tissues, and BJAB and JEG. *b*, *In situ* hybridization of *Pit-1* transcripts. *Pit-1* transcripts were present in the developing neural tube; in diencephalon (D), mesencephalon (M), rhombencephalon (R), and spinal cord (S), and telencephalon (T). Day e13 shows trace hybridization to indicated areas, whereas no hybridization was observed on e15. On e16 (and e18, not shown), hybridization reappeared exclusively in the anterior pituitary gland (AP). *c*, *In situ* hybridization of *Oct-2* transcripts showed that on e13 and e16, *Oct-2* transcripts are present in diencephalon (D), mesencephalon (M), rhombencephalon (R) and spinal cord (S). In adult rat brain, *Oct-2* transcripts were restricted to a few regions, including the suprachiasmatic (Sch) and medial mamillary (MM) nuclei. *d*, *In situ* hybridization of *Oct-1* transcripts revealed that *Oct-1* was present in the egg cylinder (EC, day e8). By embryonic day 13 trace hybridization was observed in mesencephalon (M), rhombencephalon (R) and spinal cord (S). Hybridization was shown in adult cerebellum in the granule cell layer (CERgr) and in the lymph nodes.

METHODS. As described in Fig. 2. *Pit-1* probe, which incorporates the N-terminus and part of the POU-specific domain, was transcribed with T7 RNA polymerase and produced a probe of 538 nucleotides of which 355 were protected by mature transcript. *Oct-2* probe, which includes the entire POU domain, was transcribed with T3 RNA polymerase and produced a probe of 547 nucleotides of which 440 were protected by hybridization to mature transcript. *Oct-1* probe covered the B portion of the POU-specific domain and POU homoeodomain.



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LETTERS TO NATURE

The HH111 jet and multiple outflow episodes from young stars

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HERBIG–HARO jets are shocked beams of gas escaping supersonically from new-born stars. Here I report the discovery of an exceptionally fine jet, HH111, emerging from a young star still embedded in the Orion molecular clouds. The jet system displays four bow-shock-like features, moving in pairs in opposite directions from a 25- $L_{\odot}$ infrared source. With an extent of more than 0.8 parsec, HH111 is the largest bipolar Herbig–Haro flow known. It is argued that such jets are highly transient phenomena that are related to eruptive accretion events in the build-up of the central star.

As part of a continuing search for new Herbig–Haro (HH) objects¹, deep red plates have been taken with the European Southern Observatory (ESO) Schmidt telescope of the L1617 cloud in Orion. Several small nebulae were identified, and subsequent interference-filter CCD images and long-slit spectroscopy have shown them to be genuine Herbig–Haro objects, HH110 to HH113. Of these, HH111 is a spectacular jet system, and is shown in Fig. 1 (see legend for positions). The figure is a composite of contour plots made from several CCD images. The main part of the complex is the highly collimated jet which

emerges from an embedded infrared source (IRS, the location of which is indicated in Fig. 1, which also gives identifications for individual parts of the system). Over its first 9 arcsec the jet is very faint, after which it brightens abruptly. The body of the jet consists of a large number of individual knots; from knot A to knot O its length is 50 arcsec. At the distance of the Orion molecular clouds (460 pc), this leads to a projected length of 23,000 AU or 0.11 pc, an unusually large size for a jet. Adopting an upper limit of 1.5 arcsec for the (unresolved) width of the jet, this corresponds to a lower limit of 33 for the length-to-width ratio.

Farther out there are two nebulae, P and V, which are interpreted here as bow-shocks in the flow. The first of these, P, coincides with a faint background star, and shows two faint but well defined wings stretching backwards from its apex. The second bow-shock (V) is situated 72 arcsec farther along the flow axis (138 arcsec from where the jet emerges), and has a single very bright core, again with two wings bent backwards. In addition, it has a small tail ending in two faint knots (T_1 and T_2). The distance from the second bow-shock to the energy source is 142 arcsec, corresponding to a projected distance of 65,000 AU, or 0.32 pc. It is noteworthy that the jet is actually visible between the two bow-shocks as a very faint and diffuse trail of slightly luminous gas, the brightest part being immediately after the first bow-shock (knots Q to S). On the eastern side of the source there is no counter-jet, but 200 arcsec and 226 arcsec, respectively, from the source there are two more bow-shocks (X and Y), both facing in the opposite direction of the two western bow-shocks. X is more compact, with a well

TABLE 1 Heliocentric velocities (V_{hel}), electron densities (n_e) and line intensities in HH111

Section	V_{hel} (km s ⁻¹)	n_e (cm ⁻³)	[O I] 6,300	[O I] 6,363	H α 6,563	[N II] 6,584	[S II] 6,717	[S II] 6,731	No. of pixels
D-I	-63	1,500	116	30	112	22	156	188	9
J-L	-65	500	97	22	116	18	151	142	6
M-O	-51	100	—	—	58	—	48	36	9
P	-45	< 25	—	—	—	—	73	36	4
T	-68	250	—	—	33	—	39	31	4
V	-56	500	107	36	382	31	215	196	3
X	+106	—	—	—	102	—	—	—	4
Y	+58	1,000	—	—	83	—	21	22	4

Line intensities are relative, in arbitrary units, and are summed over the number of pixels given in the last column. Sections A–V and X, Y are from two different spectra, adjusted to the same flux scale. Velocities are based on the H α and [S II] lines, weighted after their relative intensities.

defined eastern edge and a more diffuse western side, whereas Y is larger, fainter and more ill-defined. The total extent of the whole HH111 complex, from V to Y, is 367 arcsec, or in projection 169,000 AU or 0.82 pc. This makes it even larger than the bipolar Herbig-Haro complex HH46/47 (refs 2, 3).

At very low light levels a diffuse cone-shaped nebula is visible with its apex around the base of the jet. On CCD images taken through filters that exclude shock-excited emission lines, the jet disappears but the faint nebula remains. It is thus best interpreted as the stellar continuum of the embedded energy source, scattered through a cavity around the flow axis⁴⁻⁶.

Two long-slit spectra have been taken along the HH111 flow axis, one covering the jet and western bow-shocks, and the other the two eastern bow-shocks. Figure 2 shows that the jet has a characteristic low-excitation nature, whereas the bow-shock has higher excitation. Heliocentric radial velocities and relative line intensities for various sections of the flow have been measured, and are given in Table 1. The velocities, the excitation parameter (ratio of [SII] 6,717-Å emission to H α) and the electron density n_e derived from the [SII] 6,717-Å/6,731-Å ratio⁷ are plotted along the jet in Fig. 3. The jet and western bow-shocks clearly move towards us, whereas the eastern bow-shocks recede from us. The maximum velocity difference, between X and T, is 174 km s⁻¹. The heliocentric velocity of the molecular cloud from which HH111 emerges, based on CO observations, is +23 km s⁻¹.

The faint knots A to C increase in brightness with distance from the source, and this region probably represents a highly obscured section of the jet as it emerges through a steep density gradient. At D the jet suddenly brightens (perhaps on reaching the surface of the cloud), achieving a maximum brightness at knot H. Within the measurement uncertainty the velocity of the jet is constant along its brightest part, D to L. It then slows down (M to O), reaching its lowest velocity at the first bow-shock, P. This is accompanied by a dramatic decrease in electron density, possibly an indication that the jet tubus is widening along the flow. At the same time its low-excitation nature becomes less pronounced.

A cut along the axis of the second bow-shock shows considerable changes in physical conditions. The tail T emits mainly in the sulphur lines, but the bright head V is a strong H α emitter. Going from T to V the velocity decreases and the density increases. This is what one would expect at the tip of the flow, where it rams into the ambient medium. The eastern bow-shocks are more difficult to understand. The first of these, X, emits H α almost exclusively, so no density information is available (only one other such case is known, the bow-shock in HH83). X has

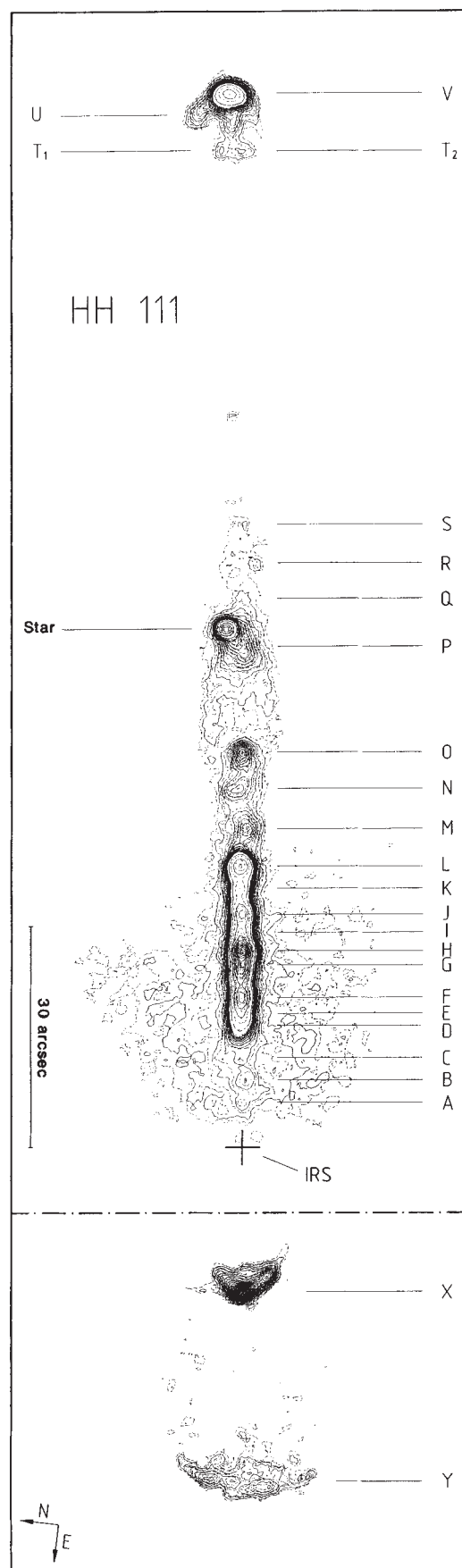


FIG. 1 A composite figure of the HH111 complex. The location of the infrared source is marked by a cross, the size of which indicates the positional uncertainty. The jet and/or bow-shocks on either side move away from the energy source at highly supersonic velocities. An empty section of about 180 arcsec separates the upper and lower parts. The upper section is a contour plot from two CCD images taken at the Danish 1.5-m telescope at ESO, La Silla, through an interference filter transmitting the [SII] 6,717-Å and 6,731-Å lines ($\lambda_c = 6,742$ Å, full width at half maximum (FWHM) = 85 Å). The image of the body of the jet is an average of three 1-h exposures, and the upper bow-shock is from a single 1-h exposure. The lower section is a similar 1-h exposure, but through an interference filter that transmits the H α 6,563-Å line and surrounding [NII] lines ($\lambda_c = 6,565$ Å, FWHM = 78 Å). In the upper section there are 21 contours starting at 114 (in arbitrary units) with steps of 3, followed by 17 contours starting at 200 with steps of 50, to outline the structure within the bright trunk of the jet. The lower section contains 50 contours with steps of 3, starting at 250 (the background is higher in H α than in [SII]). Cosmic rays have been removed and background stars erased, except for one behind the first bow-shock. Individual knots (or, on steep intensity gradients, ridges on a slope) are identified. The positions of the features and infrared source are as follows: H, 5°49'07.2", 2°47'52"; P, 5°49'04.4", 2°47'57"; V, 5°48'59.5", 2°48'06"; X, 5°49'22.1", 2°47'20"; Y, 5°49'23.8", 2°47'17"; IRS, 5°49'08.9", 2°47'50". The accuracy of the positions is ~1 arcsec, but ~5 arcsec for the IRSs.

a much higher velocity than the second, more diffuse bow-shock, Y, which has a high density.

The IRAS source 05491+0247 is located just to the east of the jet. It was detected as a rather faint near-infrared object with a K magnitude of 12.83 at the ESO 2.2-m telescope, at a location 4 arcsec east of the beginning of the jet. Combining the near-infrared photometry and data from IRAS (Infrared Astronomical Satellite), and extrapolating beyond $100\ \mu\text{m}$ with a black body peaking at $100\ \mu\text{m}$, the total luminosity of this object is $25\ L_{\odot}$. This is rather high for a Herbig-Haro energy source⁸.

CCD images taken through a filter with peak transmission around $0.95\ \mu\text{m}$ show no optical counterpart of the source, suggesting a lower limit to the colour index $m_{0.95} - m_{2.2} > 10$ magnitudes. An order-of-magnitude estimate of extinction can be made by assuming an intrinsic colour index of zero; adopting a standard extinction curve then gives $A_V > 30$ magnitudes along the line of sight. The presence of the cone-shaped reflection nebula indicates that the extinction along the jet axis is considerably smaller, probably only a few magnitudes. The ambient material of the young star is thus distributed highly anisotropically, suggesting the presence of a circumstellar disk.

Because of its very large extent and relatively small radial velocities, the jet is probably flowing almost in the plane of the sky. This is supported by detailed observations of the velocity field of a major molecular outflow, which is found to be coincident with the optical outflow (B. Reipurth and M. Olberg, manuscript in preparation). An order-of-magnitude estimate of

the dynamical age of the complex can be derived using its spatial extent and the largest observed difference in radial velocities; the result is 1,700 years if we assume a flow angle to the plane of the sky of 20° , and 800 years if we assume an angle of 10° . The mean flow velocities derived in the two cases are $500\ \text{km s}^{-1}$ and $1,000\ \text{km s}^{-1}$, respectively. The highest velocities observed in HH objects are $\sim 600\ \text{km s}^{-1}$. Clearly the HH111 complex represents a phenomenon that evolves with amazing rapidity.

It has been advocated for some time that jets from young stars are quasi-continuous phenomena⁹. There are, however, a number of observations that suggest the opposite, specifically that they are of a highly transient but repetitive nature. First, the double bow-shocks of HH111 on each side of the jet strongly suggests that several pulses have been sent out from the star within a rather short time. Evidence for such multiple eruptions is also seen in some other HH systems, such as HH28/29, HH46/47, HH80/81 and Th 28 (refs 10, 2, 1 and 11 respectively). Second, when obvious bow-shocks are found, jets are not necessarily also observed; the best case of this is the HH100/101 complex¹², and HH45 is also a likely case¹³. Third, energy sources of HH objects are found to be very inhomogeneous, in particular displaying a large spread in luminosity⁸. One energy source, related to HH57, has been seen to erupt into an FU Orionis star^{14,15} (see later), and the energy source of HH28/29 has also been identified as presently undergoing an FU Orionis outburst^{10,16}. The energy source of HH46/47 has been observed to be strongly variable⁵. These observations suggest that the driving sources can change on timescales much shorter than the dynamical timescales of HH objects. Finally, if jets are of an intermittent nature, then some infrared sources (IRS) ought to be found that do not show any shocked outflow but that are

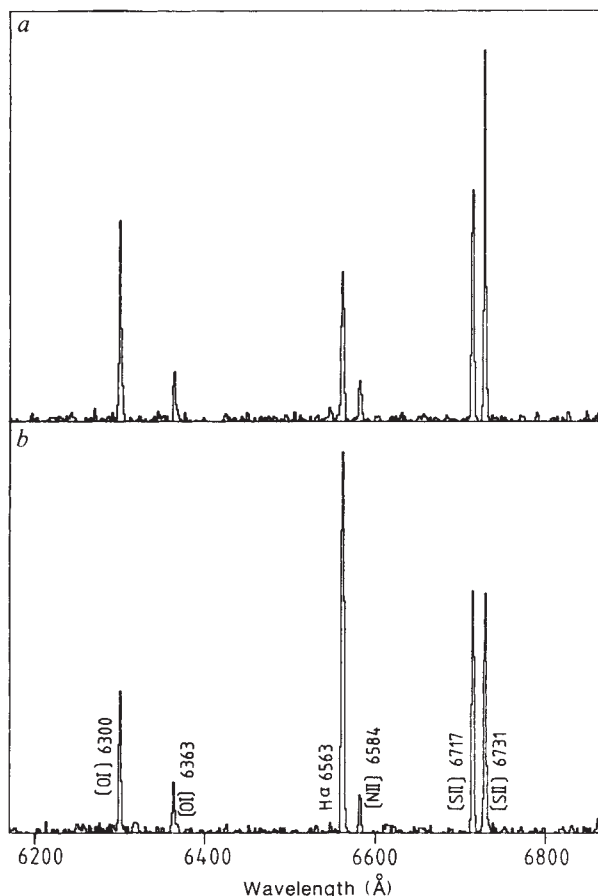


FIG. 2 Two sections of a long-slit spectrum taken along the HH111 flow axis, using the ESO 2.2-m telescope and a Boller and Chivens spectrograph with an RCA CCD detector. The dispersion was $1.75\ \text{\AA}$ per pixel, and a slit width of $2.3\ \text{arcsec}$ was used. The spatial resolution along the slit was $1.76\ \text{arcsec}$ per pixel. *a*, Spectrum of knots D to I, comprising the brightest part of the jet. The strength of the sulphur lines relative to $\text{H}\alpha$ reveals the low-excitation character of the jet, and the sulphur line ratio yields a high electron density. *b*, Spectrum of the bright core of the bow-shock denoted V in Fig. 1. Compared with the jet spectrum above, the bow-shock is clearly of higher excitation and lower density.

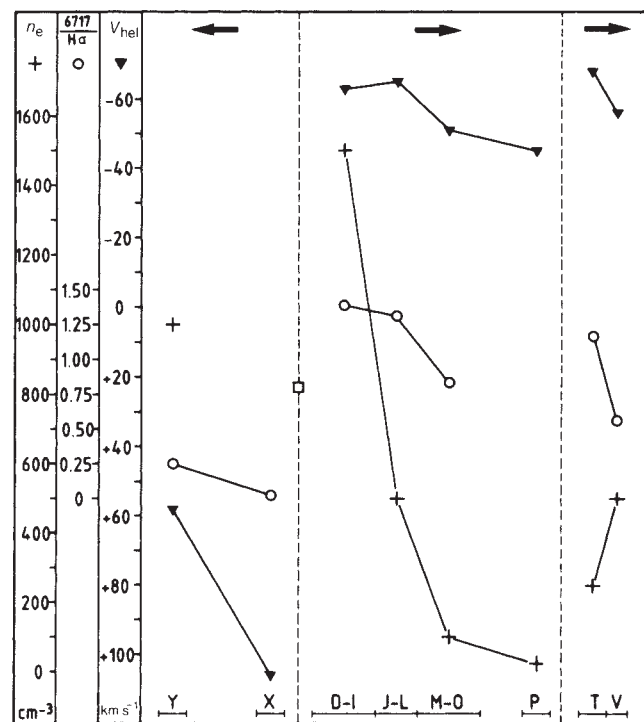


FIG. 3 Physical conditions along the HH111 complex. The electron density n_e (+), excitation ($\circ$) and heliocentric radial velocity V_{hel} ($\blacktriangledown$) have been determined from long-slit spectroscopy. The sections of the slit used to derive individual points are marked at the bottom, with indications of the contributing knots. Flow directions are indicated at the top. The two right-hand (western) sections have high negative radial velocities, whereas the left (eastern) section shows high positive velocities. The CO cloud velocity is marked by $\square$. The electron density drops markedly along the body of the jet. It appears that the western bow-shock (right section) shows changes within only $6\ \text{arcsec}$: the flow decelerates when it rams into the ambient medium, whereas the density increases and excitation becomes higher (corresponding to a lower $6,717/\text{H}\alpha$ index).

otherwise similar to sources such as, for example, HH46/47 IRS, HH83 IRS and HH111 IRS. Several sources associated with infrared reflection nebulae (and thus with an opening to the cloud surface) appear to be in a similar stage, with luminosities and energy distributions comparable to the above mentioned jet energy sources. Examples are Re 5 and IRN (refs 17–19).

Although each of these observations can be interpreted in several ways, as a whole they lend support to the conjecture that jets are intermittent phenomena, which turn on and off many times during the earliest phases of a new-born star. This view is consistent with our present observational and theoretical understanding of young low-mass stars. Many of the observed properties of T Tauri stars can be best understood by assuming that they have circumstellar disks^{20–22}. The sudden FU Orionis eruptions in which young stars brighten by 5–6 magnitudes,

followed by a slow decline²³, have been successfully modelled as rapid-accretion events in such disks²⁴. FU Orionis stars are observed to produce massive, supersonic winds^{25,26}, which are likely to have profound effects on their surroundings. It is noteworthy that the two most embedded FU Orionis stars known, HH57 IRS and L1551 IRS, are both associated with HH objects^{14–16}. There is still no consensus about the detailed mechanisms by which the gravitational potential energy of the circumstellar material is used to produce collimated outflows^{27,28}, but in all cases those proposed involve disk accretion.

The results presented here suggest that the two observational phenomena, FU Orionis eruptions and Herbig–Haro objects, are closely related, and that shocked jets are repetitive eruptive events. □

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Can solid-state effects enhance the cold-fusion rate?

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RECENTLY two groups^{1,2} have reported finding experimental evidence for an unexpectedly high rate of nuclear fusion at room temperature during the process of electrolytic deposition of deuterium on palladium^{1,2} and titanium². To achieve the rate of neutron production ($\sim 10^{-23} \text{ s}^{-1}$ per deuteron pair) cited in ref. 2 (and *a fortiori*, that inferred in ref. 1) requires the solid-state environment to produce either an unusual enhancement of the fusion reaction rate or a large suppression of the Coulomb barrier between deuterons. The latter would presumably arise from some kind of sophisticated many-body screening effect in Pd or Ti, perhaps associated with quasiparticles of large effective mass². Here we point out that within the framework of the lowest-order Born–Oppenheimer approximation, a very severe constraint is imposed on all such screening mechanisms in solids in equilibrium by the observable binding affinity of ⁴He atoms for the metal in question. Unless the latter is quite anomalous, the Coulomb barrier penetration in a solid in equilibrium cannot be enhanced anywhere near the magnitude required to explain the fusion rates inferred from the experiments.

The theoretical rate, Γ , of neutron production by fusion per second per deuteron pair is of the form $\Gamma = Aa^{-3}e^{-B}$, where A is the rate of the fusion reaction, $d + d \rightarrow {}^3\text{He} + n$, per unit probability density at $r=0$, r is the relative separation of the two deuterons, a^3 is approximately the volume of classically accessible motion for the pair, and e^{-B} is a Coulomb barrier penetration factor^{3–5}. The fusion rate is $\approx 1.5 \times 10^{-16} \text{ cm}^3 \text{ s}^{-1}$, a generous lower order-of-magnitude limit on a in any solid-state environ-

ment is $0.1 \text{ \AA}$, and thus the experiments would require $B < 80$. For the D_2 molecule in free space⁵ $B \approx 175$, but both theory⁴ and experiment⁶ suggest that much smaller values can be obtained when the deuterons are bound together by a muon or other heavy particle, which effectively screens the nuclear Coulomb charges from one another down to very small distances. A heavy-electron quasiparticle is unlikely to produce the required screening on sub-ångström scales, because such quasiparticles have an extended structure in space; the effective mass of the electrons on such scales is the bare electron mass. On the other hand, one might suspect the presence of short-range screening effects associated with the large electron densities in the metal.

The essential point of our argument is that if the effective repulsion of two deuterons at short distance is substantially weakened by solid-state effects, then these effects should also lead to greatly increased binding of an α -particle to the metal. To make the argument precise, we define, within the lowest-order Born–Oppenheimer approximation, the effective ‘potential energy’ $V(\mathbf{R}, \mathbf{r})$ of two deuterons placed in the metal at $\mathbf{R} + \mathbf{r}/2$ and $\mathbf{R} - \mathbf{r}/2$ respectively. (We assume that two additional electrons are added to the metal, to preserve overall charge neutrality; as these electron energies cancel out in the argument we do not consider them explicitly.) We split V into three contributions: (1) the direct Coulomb interaction, e^2/r , of the two deuterons with each other; (2) the Coulomb interaction, $V_c(\mathbf{R} + \mathbf{r}/2) + V_c(\mathbf{R} - \mathbf{r}/2)$, of the two deuterons with the environment, that is, with all other charges, electronic and ionic; and (3) everything else, namely the kinetic energies and mutual interactions of the environment, which we call K . Here

$$V_c(\mathbf{R}) = e \int d^3r \frac{\rho(\mathbf{r})}{|\mathbf{r} - \mathbf{R}|} \quad (1)$$

where $\rho(\mathbf{r})$ is the charge density (expectation value) of the environment.

Consider now the 'chemical energy' $U(\mathbf{R}+\mathbf{r}/2)$ of an α -particle placed at $\mathbf{R}+\mathbf{r}/2$, that is, its potential energy relative to that of an α -particle placed at infinite distance from the metal. If we use the exact environment wavefunction for the ground state of the two-deuteron problem as a variational wavefunction for the α -particle problem, noting that the α -particle has charge $2e$, we see that $U(\mathbf{R}+\mathbf{r}/2) \leq K + 2V_c(\mathbf{R}+\mathbf{r}/2)$, with a similar inequality at $\mathbf{R}-\mathbf{r}/2$. Adding the two inequalities we find, for all $\mathbf{R}$ and $\mathbf{r}$, that

$$V(\mathbf{R}, \mathbf{r}) \geq \frac{1}{2} \left[U\left(\mathbf{R} + \frac{\mathbf{r}}{2}\right) + U\left(\mathbf{R} - \frac{\mathbf{r}}{2}\right) \right] + \frac{e^2}{r} \quad (2)$$

In the limit of zero temperature, which we consider initially, the actual energy eigenvalue E_{2d} of the deuteron pair is bounded above by the energy of two deuterons at infinite separation in the metal, which is $-(E_d + K_d)$, where $E_d = 1$ Ry is the binding energy of deuteron in free space (Ry is the rydberg) and K_d is its affinity to the metal. Similarly, $U(\mathbf{R} \pm \mathbf{r}/2)$ is bounded below, for all $\mathbf{R}$ and $\mathbf{r}$, by $-(E_4 + K_4 + E_{zp}^\alpha)$, where $E_4 = 5.802$ Ry and K_4 are, respectively, the free-space binding energy and affinity of the ^4He atom, and E_{zp}^α is the zero-point energy of the α -particle in the metal, that is, its energy relative to the minimum value of the potential $U(\mathbf{R})$. Inserting these inequalities into (2) we find

$$V(\mathbf{R}, \mathbf{r}) - E_{2d} \geq \frac{e^2}{r} - \lambda \frac{e^2}{a_0} \quad (3)$$

where $\lambda = \frac{1}{2}[E_4 + K_4 + E_{zp}^\alpha - 2(E_d + K_d)]/\text{Ry}$, and a_0 is the usual Bohr radius. The inequality (3) is rigorous and applies to arbitrary position of the two deuterons, whether in the bulk or on the surface.

Clearly, the exponent B for penetration to $\mathbf{r}=0$ is bounded below by its value for the potential on the right-hand side of (3). Furthermore, it is adequate for the present purpose to evaluate the latter by the simple WKB approximation⁴. Thus, taking into account the reduced mass of the deuteron pair, we can write

$$B \geq B_0(\lambda) \equiv 2 \int_0^{a_0/\lambda} dr \left(\frac{M_d e^2}{\hbar^2} \right)^{1/2} \left(\frac{1}{r} - \frac{\lambda}{a_0} \right)^{1/2} \\ = \pi \left(\frac{M_d}{m_e} \right)^{1/2} \lambda^{-1/2} \approx 189 \lambda^{-1/2} \quad (4)$$

where m_e is the mass of the electron and M_d is that of the deuteron.

The deuterium affinity, K_d , is⁷ 0.17 Ry in Pd and presumably of similar size in Ti; to the best of our knowledge there is no direct measurement of the helium-atom affinity, K_4 , for Pd or Ti, but the fact that ^3He desorbs from Ti at room temperature⁸ suggests that, as for other metals, K_4 is either positive or very small ($\ll 1$ eV). Similarly, the zero-point energy of the α -particle has not been measured, but it should be bounded above by that of the deuteron; the latter can be estimated from the isotope dependence of K for hydrogen⁷ to be $\ll 1$ eV. If we neglect K_4 and E_{zp}^α we find $\lambda \approx 1.78$ and hence $B \geq 141$. This value corresponds to a fusion rate of $\sim 10^{-50} \text{ s}^{-1}$ per deuteron pair, 27 orders of magnitude below that inferred in ref. 2. To reduce B to the maximum value (~ 80) consistent with the fusion rates in ref. 2 would require $K_4 + E_{zp}^\alpha$ to be ~ 100 eV, a quite incredible value which would have other easily observable consequences. The bound given here is probably orders of magnitude above the exact barrier-penetration probability, as one can see by applying the method to the free D_2 molecule, where it gives a rate of $\sim 10^{-53} \text{ s}^{-1}$, which is about ten orders of magnitude too fast.

Unless the potential $V(\mathbf{R}, \mathbf{r})$ is itself affected on a scale of a few electron volts by room temperature, which seems most unlikely, a simple extension of the above analysis to finite temperature with a Maxwell-Boltzmann (or Bose) distribution for the deuterons shows that the lower bound on B at room temperature is negligibly different from that quoted above.

The present argument cannot, strictly speaking, at present exclude mechanisms that rely essentially on the presence of large numbers of 'third-party' deuterons (because there is no measurement to date of helium affinities under these conditions), nor those that rely on some unspecified highly non-equilibrium energy distribution which cannot be mimicked by a thermal distribution with a temperature of the order of room temperature. Once such a mechanism is specified in detail, however, arguments similar to the above could be used to estimate its maximum efficacy. We have now extended these arguments beyond the lowest Born-Oppenheimer approximation⁹. $\square$

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Origin of precursors of organic molecules during evaporation of meteorites and mafic terrestrial rocks

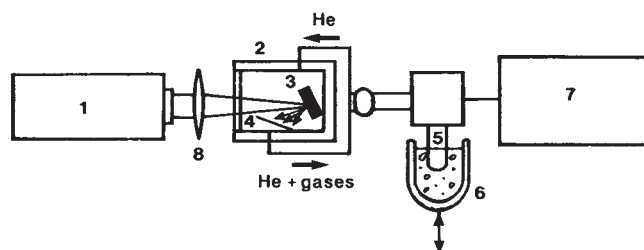
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THE accretion of the Earth was characterized by high-temperature transformations of planetesimal matter during high-velocity impacts. In the final stages of the Earth's growth, up to 30% of planetesimal mass and the corresponding mass of the target were evaporated¹⁻³. Here we look at the effect of laser-pulse heating on meteorite materials and silicates in order to simulate the vaporization which occurs during impacts and to study the chemical composition of the gases produced. In our experiments, the residual gas mixture consisted of both oxidized and reduced components: CO , CO_2 , SO_2 , H_2O , H_2 , N_2 , H_2S , COS , CS_2 , various hydrocarbons from C_1 to C_6 , HCN and CH_3CHO . In addition, we find that the composition of the gas mixtures is qualitatively similar for all samples. Our data give an idea of the chemical composition of gases that may have been released into the Earth's early atmosphere by impact devolatilization. In the gas mixtures produced by these experiments we measured weight per cents of $\sim 10^{-3}$ for HCN , $\sim 10^{-4}$ for CH_3CHO and $\sim 10^{-2}$ for hydrocarbons. Impact vaporization is no less effective for the formation of HCN than is the formation of HCN by impact reprocessing of the atmosphere⁴. For the production of aldehyde and hydrocarbons, impact vaporization is even more effective. The gas mixtures formed by vaporization of silicates provide favourable conditions for abiotic synthesis of organic compounds.

Impact processes may have been important in the formation of the Earth's atmosphere and hydrosphere as it has been convincingly demonstrated that the Earth's atmosphere formed simultaneously with its growth⁵⁻⁷. Volatiles were released by the melting and vaporization of planetesimal matter during impacts, but direct experimental simulations of large-scale impact processes at impact velocities ($> 8 \text{ km s}^{-1}$), with *in situ* analysis of released gases is impossible. We therefore used the effect of

FIG. 1 Experimental apparatus: 1, Nd YAG laser; 2, hermetic cell; 3, sample; 4, foil for condensate collection; 5, cold trap; 6, liquid nitrogen Dewar flask; 7, chromatograph/mass spectrometer; 8, focusing lens.



laser-pulse heating on various (ultra)mafic rocks and meteorite material to simulate the high-temperature vaporization which occurs during high-velocity impacts. An important feature of vaporization processes, when compared with degassing from melts, is the thermal dissociation of crystal silicate matrices which provides a relatively dense vapour cloud above the melt. For laser-pulse heating, with power density $>10^6 \text{ W cm}^{-2}$, and pulse duration $>10^{-5} \text{ s}$, the conditions near the evaporating surface yield a silicate vapour in thermodynamic equilibrium which should simulate conditions during large-scale impact vaporization⁷. The difference, therefore, in the chemical composition of the vapour phase for our laser experiment and for actual impacts will be defined by the difference in the chemical quenching temperatures during the expansion of the vapour cloud. According to the saturated vapour law the relative concentrations of major gases in the vapour cloud during expansion are weakly temperature dependent^{7,8}. We will therefore assume that the gases released by laser vaporization and impact vaporization are similar.

Our experimental set-up is schematically represented in Fig. 1. The sample was mounted in the hermetic stainless-steel cell which has a quartz-glass window transparent to the laser beam. In one series of runs the cell was filled with helium while in another series the cell was filled with hydrogen at atmospheric pressure and room temperature. The samples we used include augite, peridotite, picritic gabbro with pyrite inclusions, basalt and L5 ordinary chondrite (Tsarev) and C30 carbonaceous chondrite (Kainsaz) meteorites. Before mounting the samples, the surface of each was cleaned to a depth of $\sim 0.5 \text{ mm}$ using a dry abrasive corundum disk. Subsequently, a weak laser pulse with a power density of $10^3\text{--}10^4 \text{ W cm}^{-2}$ was used to remove adsorbed gases including possible organic contamination on the surface. Purging of the cell was continued until the blank experiment (without the laser pulse) gave a chromatogram with carbon- and sulphur-containing gases below the detection limit of the instrument. The sample was exposed to a Nd YAG laser

pulse (wavelength $1.06 \mu\text{m}$ and duration $\sim 10^{-3} \text{ s}$). The power density in the beam was varied in the range of $10^6\text{--}10^8 \text{ W cm}^{-2}$ by changing the diameter of the focusing spot from 1 to 10 mm. The energy in the pulse was $\sim 300 \text{ J}$. As a result, the temperature at the evaporating sample surface was 3,500–4,500 K. The loss of 7–20 mg of the sample mass during a laser pulse (the sample was weighed before and after the experiment) was the result of both vaporization and dispersion of melted droplets from the crater that was created in the sample by the laser pulse. Each time, a fog of condensed particles filled the interior of the cell following the laser pulse. About 10–20 mg of melt remained on the crater walls. So, during the experiment, gases were released in the vapour cloud from vaporized, melted (both dispersed and residual) and heated solid material in the vicinity of the crater. Thus, the gases are released from a mass several times larger than the measured mass loss. Nevertheless, the mass loss gives a good indication of the mass of highly heated sample material. The gas mixture that formed in the chamber after each laser pulse was concentrated on two sequential traps cooled at liquid-nitrogen temperatures before chromatographic analyses. One trap was filled with glass microspheres, the other trap with activated coal in order to retain H_2 and N_2 . An LKB 9000 gas-chromatograph-mass-spectrometer was used to analyse the pre-concentrated gas mixtures. In the chromatographic part of the instrument, two columns were mounted: a column packed with 5-Å molecular sieves was used to analyse volatiles and another column packed with Porapak Q was used to analyse the less volatile compounds. The results of analyses of the residual gas mixture formed after laser evaporation of various samples are given in Table 1.

It can be seen that the gas mixtures are composed of both oxidized and reduced components (Table 1). Gas mixtures from different samples in the helium atmosphere qualitatively resemble each other. This is because in an inert atmosphere, carbon and sulphur mainly evolve as oxides (CO , CO_2 , SO_2) irrespective of the fact that, in some samples (for example,

TABLE 1 The results of some typical analyses of the chemical composition of residual gases

Sample	Initial concentration (wt%)		Mass loss (mg)	Gases ($\times 10^{-6} \text{ g}$)										CO	
	C	S		H_2	N_2	CO	CO_2	HC†	SO_2	H_2S	HCN	CH_3CHO	CO_2	CO + CO_2	HC
Detection limit				0.2	0.1	0.1	0.02	0.02	0.1	0.08	0.06	0.02			
He atmosphere															
Augite	ND	ND	10.8	<0.2	0.4	3.3	2.7	0.7	<0.1	<0.08	<0.06	<0.02	1.2	8.6	
Basalt	0.04*	<0.01*	20.1	<0.2	0.5	3.5	5.9	0.6	<0.1	<0.08	<0.06	~ 0.02	0.6	16	
Peridotite	0.03*	0.03*	10.2	2.6	0.1	25.4	21.4	1.3	ND	ND	ND	ND	1.2	36	
Gabbro	0.05*	3.04*	14.0	19.6	0.9	36.3	28.3	2.6	240	1.8	0.7	0.2	1.3	25	
L5 (Tsarev)	ND	1.92 ¹¹	7.3	6.2	1.9	36.3	19.8	2.5	17.7	ND	0.2	~ 0.08	1.8	22	
C30 (Kainsaz)	0.61 ¹³	2.06 ¹²	10.5	18.6	1.9	236	94.3	12.2	<0.1	ND	ND	~ 0.04	2.5	27	
H ₂ atmosphere															
Augite			9.1	ND	ND	25.6	9.0	8.2	<0.1	<0.08	<0.06	<0.02	2.8	4.2	
L5 (Tsarev)			7.1	ND	ND	16.9	10.8	7.0	<0.1	162	ND	~ 0.1	1.6	4.0	
C30 (Kainsaz)			9.7	ND	ND	266	46.4	25.6	<0.1	149	0.4	~ 0.2	5.8	12	

ND, not determined.

* Certified by IGEM Acad. of Sci. USSR.

† HC, the sum of hydrocarbons.

meteorites), carbon and sulphur were present in their reduced state. Oxidation processes, which dominate the chemistry of the vapour cloud, are the result of molecular and atomic oxygen in the cloud produced by thermal dissociation of silicate minerals in our samples^{9,10}. The volume mixing ratio of molecular and atomic oxygen in the cloud is estimated at $\sim 30\%$ ⁷. Oxygen actively interacts with condensed particles and it is almost completely absorbed during cooling. Significantly, oxides of carbon (CO and CO₂) were also the main carbon-containing compounds resulting from vaporization of samples in an H₂ atmosphere. Moreover, for different types of meteorites and rocks the CO/CO₂ ratios agree to within one order of magnitude, irrespective of the nature of the atmosphere (He or H₂) in which vaporization was performed. The ratio of the sum of oxides, CO+CO₂, to the sum of hydrocarbons and the relationship between hydrocarbons are also reproducible within one order of magnitude (Fig. 2). The experiments in an H₂ atmosphere, when compared with those in an inert atmosphere, resulted in both cases in the greater formation of carbon oxides relative to hydrocarbons and comparable patterns of distribution of hydrocarbons (although a slight increase in CH₄ production is evident from Fig. 2). This observation suggests the formation of hydrocarbons by quenching in a hot inner part of the vapour cloud which is not mixed with the ambient gas (H₂ or He). By contrast, the formation of sulphur-containing gases proceeds even in parts of the vapour cloud that are mixed with the ambient gas and results in formation of mainly H₂S in the hydrogen atmosphere, compared with the formation of SO₂ in the He atmosphere (Table 1). Laser pulses with a power density of 10^7 – 10^8 W cm⁻² produced a significant amount of hydrocarbons but when the intensity of the laser pulse was decreased the hydrocarbon output also decreased. Specifically, for laser pulses with power

densities $<10^6$ W cm⁻² the amount of hydrocarbons was below the detection limit of the instrument, whereas the release of the other major gases did not change. We suggest that hydrocarbons are indeed formed inside a hot and dense vapour cloud and not by pyrolysis of organic matter that is already present in the samples. Significantly, the majority of the hydrocarbons are non-saturated. It is important to note that, together with hydrocarbons, carbon-containing compounds with the heteroatoms—HCN and acetaldehyde—were measured. The release of water vapour in the experiments was detected but quantitative measurements of water were uncertain because of its strong adsorption. A small amount of N₂ was also detected.

The qualitative reproducibility of gas mixtures of this composition suggest that our experimental data may be used to evaluate the chemical composition of the early Earth's atmosphere. If one assumes that impact-evaporative processes were the main source for gases in the Earth's atmosphere in its final stages of formation, then the major gases released would be the oxides, CO, CO₂, SO₂, H₂O, molecular nitrogen and a certain non-negligible quantity of reduced species. The removal rate of certain components (for example, H₂, CO₂ and SO₂) could be high enough to allow a shift in atmospheric composition in favour of other components, but a highly reduced composition for the primordial atmosphere seems unlikely. Impact-generated gases are favourable for abiogenic synthesis of rather complex organic compounds under the action of different inputs of energy. In particular, the occurrence in these mixtures of saturated, non-saturated and aromatic hydrocarbons together with HCN and acetaldehyde indicates the possibility of enhanced abiogenic synthesis.

Concentrations of HCN and acetaldehyde in our experiments are $\sim 0.1\%$ by weight of the sum of the major components. The total amount of gases released during evaporation of ~ 10 mg of the L5 ordinary chondrite Tsarev is ~ 0.15 cm³ STP. This implies a yield of $\sim 1.5 \times 10^{17}$ molecules of HCN and acetaldehyde per gram of impact-evaporated matter. Assuming a mass of 10^{17} g for the large meteorite that caused shock heating of the Earth's primordial atmosphere, Fegley *et al.*⁴ have estimated that 10^{33} HCN molecules would have been formed in an atmosphere composed of CO, CO₂, N₂ and H₂O, and 10^{37} HCN molecules would have been formed in an atmosphere composed of CH₄, CO and N₂. The respective yield of formaldehyde in the model of Fegley *et al.* is 4–5 orders of magnitude lower than the yield of HCN. Obviously, the production of HCN in our experiments is almost insensitive to the atmospheric pressure. Based on our results, complete vaporization of a meteorite of mass 10^{17} g yields $\sim 10^{34}$ molecules of HCN and an amount of acetaldehyde a factor of five lower than that. The production of HCN during vaporization is comparable to its production by shock heating of a dense atmosphere⁴ but there are noticeably larger amounts of aldehydes and hydrocarbons formed. It is clear that estimates of realistic production of organic molecules and their precursors during large-scale impact process needs a detailed account of scaling effects. Nevertheless, we argue that impact-evaporative processes should be taken into account as a possible new source for organic matter and its precursors on the early Earth. □

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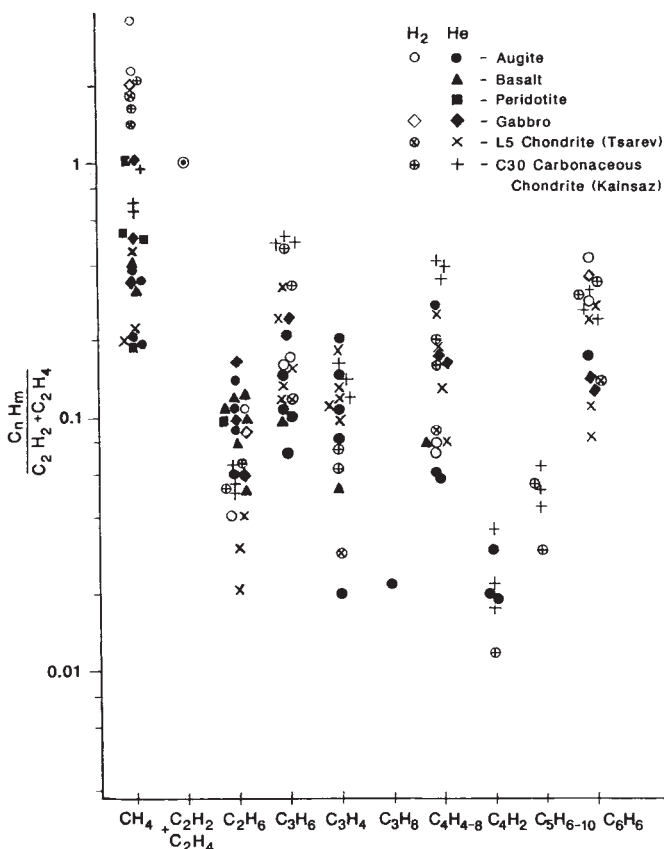


FIG. 2 Hydrocarbons obtained during vaporization of selected samples in 26 experimental runs in He and H₂ atmospheres. For each run, the amounts of various hydrocarbons (C_nH_m) are presented relative to the sum C₂H₂ + C₂H₄. When the number of results plotted for a given hydrocarbon is less than 26 it means that the sample was either not analysed for this hydrocarbon or that the amount of the hydrocarbon was below the detection limit.

Land-sea correlations in the Pleistocene based on isoleucine epimerization in non-marine molluscs

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THE correlation between the oxygen isotope stratigraphy of the oceans and continental stratigraphic records is one of the main challenges for Quaternary research. Most continental classifications, however, are based on techniques and hypotheses that predate recent advances. Nevertheless, the deep-sea oxygen isotope global stratigraphic framework has been extended to the continents by means of some long sequences¹; but these are exceptional and regional geology is unsatisfactorily classified for correlation with the oxygen isotope signal. Because successive geological events produced similar evidence (*homotaxis*), geochronometric dating is essential for correlation, but available methods are highly site-, regional- or sample-specific. Fortunately non-marine molluscs are ubiquitous in time and space, and here we use the time-dependent epimerization of L-isoleucine in these fossils to subdivide the Pleistocene of the British Isles, and to identify more events than recognized by the existing classification². By calibrating the relative aminostratigraphic scale with independent dating methods we have set up a geochronology which is the basis for land-sea correlations back to oxygen isotope stage 15.

The standard classification of the British Pleistocene has mainly evolved since 1950 and was formally adopted in 1973 (ref. 2). Its basis was the identification of fixed-points in time, each of which had a characteristic vegetational history. The 'glacial' Pleistocene of lowland England is subdivided by three such events, the Cromerian, Hoxnian and Ipswichian, which were defined as Standard Stages². In characterizing their vegetational signatures it was not possible to use either first appearance or extinction criteria for the fossil taxa, so that the assemblage flora of each event were controlled not by evolution, but by climate. Thus, because of the homotaxial nature of Pleistocene flora, it was proposed³ that the classification was probably oversimplified. Here we apply amino-acid geochronology to address this problem.

The time-dependent epimerization of the protein amino acid L-isoleucine to its non-protein diastereoisomer D-alloisoleucine [D/L], as measured in non-marine mollusca, collected from specific stratigraphic units, is the basis of the age estimates. The ratio of D-alloisoleucine to L-isoleucine in modern non-marine molluscs is zero, but increases with the increasing age of the fossils. The temperature term in isoleucine kinetics can be eliminated because the samples were collected from localities in southern Britain that all experienced the same temperature fluctuations. It is unlikely that epimerization rates were constant so that the extent of that process at any time is the integration of all the reaction rates that have occurred in a particular sample. Our data show that it is reasonable to assume that epimerization took place at more or less the same rate in all the genera used in this investigation (Table 1). Measurements were made using routine high-performance liquid chromatography⁴ (HPLC) at the Aberystwyth and London amino-acid geochronology laboratories, with inter-laboratory comparison at the Institute of Arctic and Alpine Research, University of Colorado, Boulder.

Unlike measurements on the larger marine shells used in amino-acid geochronology investigations along the north European⁵ and other coastlines, the analysis of smaller, thin-walled non-marine shells, is less straightforward. Cleaning small shells for analysis is more difficult and, because of the relatively low concentrations of amino acids in non-marine molluscs, a proportionately larger amount of CaCO₃ has to be injected on to the ion-exchange column, leading to rapid resin degradation and unreliable resolution of the amino acids. Our strategy has been to reject all analyses with poorly resolved amino acids, or with high peak-area/peak-height ratios, wherever possible to use at least three shells from each stratigraphic unit, and, subject to availability, to re-run samples if one standard deviation exceeds 10% of the mean value. These stringent requirements have been fulfilled for most sites, but not all, because of the unavailability of large samples. But even after such precautions, some ratios do not follow a stratigraphic order: for example, at West Runton, Norfolk, one set of ratios points to a younger aminostratigraphic age than that proposed here. Measurements of a larger number of shells, however, give ratios consistent with stratigraphic position. The reason for this is unknown, but could be because of 'diagenetic artefacts'⁶, which include analytical complications, sample contamination during diagenesis or analysis which lowers the D/L ratio, diagenetic alteration of the amino-acid mixture which affects specific epimerization rates, leaching of free amino acids, or mixing of samples of different ages during sedimentation.

As with the marine molluscan investigation⁴ it is possible to recognize reworking on the basis of mixed populations. Such mixed populations are highly likely in riverine situations, as

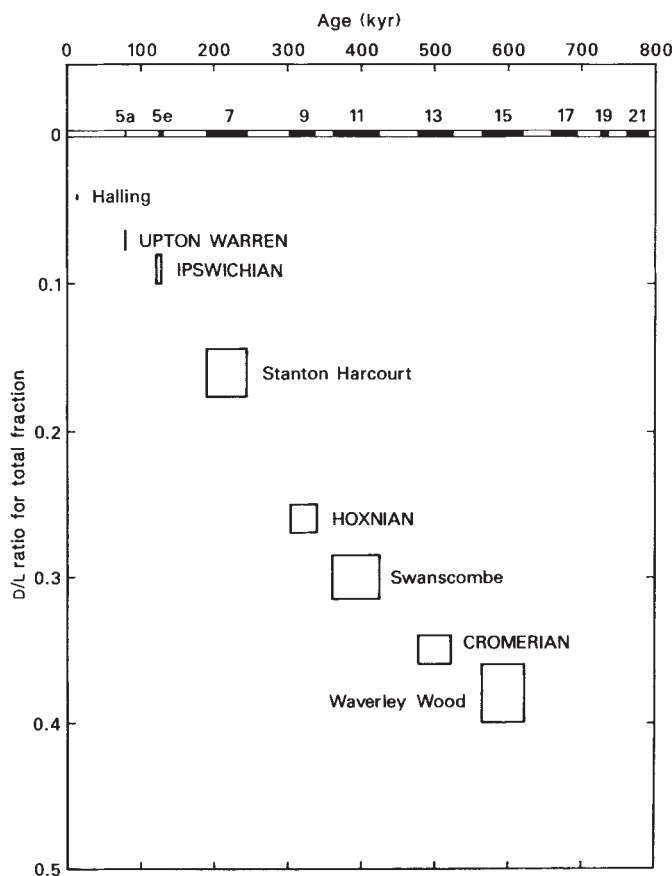


FIG. 1 Correlation of D-alloisoleucine/L-isoleucine non-marine events with odd-numbered oxygen isotope stages as tuned to orbital timescales^{16,17}. The vertical extent of the rectangles represents one standard deviation, and the horizontal extent is the duration of the oxygen isotope stage with which correlation is made.

TABLE 1 D-Alloisoleucine/L-isoleucine ratios from non-marine fossil molluscs

Site	Stratigraphic unit (ref.)	Bithynia	Lymnaea	Trichia	Cepaea
Halling	Layer G (11)			0.036 ± 0.001 (3)	
Upton Warren	Band 3 (10)		0.066 ± 0.007 (5)		
Bobbitshole	Beds B & C (21)	0.09 ± 0.14 (4)	0.1 ± 0.014 (2)		
Trafalgar Square	Grey-brickearth (12)	[0.11 ± 0.005 (5)]		0.113 ± 0.005 (3)	0.094 ± 0.004 (3)
Tattershall Castle	Detritus mud (14)			0.116 (1)	0.091 ± 0.005 (3)
Tattershall Thorpe	Detritus mud (14)				0.125 ± 0.02 (4)
Block Fen	Detritus mud (12)	0.12 ± 0.01 (3)			
Bacon Hole	Sandy cave earth (12)				0.122 ± 0.02 (5)
Old Fallow	Grey marl (22)				
Stanton Harcourt	Channel beds below SH Gravel (9)	0.154 ± 0.007 (3)	0.153 ± 0.014 (6)		
Aveley	Organic silts & clays (12)	0.148 ± 0.016 (7) [0.148 ± 0.006 (4)]	0.172 (1)		
Crayford	British Museum collection (12)	0.170 ± 0.02 (12)			
Stutton	Brickearth (23)	0.2 ± 0.034 (4)		0.177 (1)	
Portland	Head (12)			0.197 ± 0.02 (4)	
Ailstone	Bed A (22)				
West Wittering	Bed A (24)	0.139 ± 0.007 (2)	0.141 ± 0.01 (5)		
Selsey	Bed I (24)		0.164 ± 0.016 (5)		
Hoxne	Layer e (25)				
Hatfield	Grey silt (26)		0.22 ± 0.02 (5)		
Woodston	Basal beds (27)	0.243 ± 0.03 (9)		0.236 ± 0.027 (4)	0.253 ± 0.017 (3)
Ilford	Shelly bed (12)	0.23 ± 0.02 (5)			
Bushley Green	Basal beds (22)			0.235 ± 0.01 (2)	
Swanscombe	Upper middle Gravel (8)	0.312 ± 0.017 (4)			
	Lower middle Gravel (8)	0.296 ± 0.01 (4) [0.29 (1)]			
	Lower Gravel (8)	0.30 ± 0.015 (5)			
	Lower Loam (8)				[0.3 ± 0.04 (3)]
Ingress Vale	Gravel (12)				
Little Thurrock	Brickearth (28)	0.29 ± 0.024 (4)			
Clacton	Channel beds (12)				
Sugworth	Organic beds (29)				
Nar Valley	Freshwater sands (12)				
West Runton	Freshwater bed (30)	0.38 (1)			
Little Oakley	Channel deposit (*)				
Purfleet	Shelly Gravel (12)	0.34 ± 0.24 (2)			
Yew Tree Farm	Freshwater beds (31)				
Waverley Wood	Organic beds (7)			0.381 ± 0.024 (3)	

Site	Stratigraphic unit (ref.)	Gyraulus	Corbicula	Pisidium	Valvata
Halling	Layer G (11)				
Upton Warren	Band 3 (10)				
Bobbitshole	Beds B & C (21)				
Trafalgar Square	Grey-brickearth (12)				
Tattershall Castle	Detritus mud (14)				0.127 (1)
Tattershall Thorpe	Detritus mud (14)				
Block Fen	Detritus mud (12)				0.117 ± 0.016 (2) [0.126 ± 0.03 (5)]
Bacon Hole	Sandy cave earth (12)				
Old Fallow	Grey marl (22)			0.116 ± 0 (2)	
Stanton Harcourt	Channel beds below SH Gravel (9)		0.163 ± 0.016 (9)		0.174 ± 0.014 (8)
Aveley	Organic silts & clays (12)				
Crayford	British Museum collection (12)		0.187 ± 0.007 (3)		0.185 ± 0.018 (9)
Stutton	Brickearth (23)		0.17 ± 0 (2)		0.153 ± 0.004 (3)
Portland	Head (12)				
Ailstone	Bed A (22)				0.167 ± 0.004 (3)
West Wittering	Bed A (24)		0.164 ± 0.014 (7)		
Selsey	Bed I (24)		0.165 ± 0.019 (10)		0.154 ± 0.02 (4)
Hoxne	Layer e (25)				0.261 ± 0.01 (4) [0.243 ± 0.023 (3)]
Hatfield	Grey silt (26)	0.246 ± 0.002 (2)			0.247 ± 0.02 (2)
Woodston	Basal beds (27)				
Ilford	Shelly bed (12)				
Bushley Green	Basal beds (22)				0.22 (1)
Swanscombe	Upper middle Gravel (8)				
	Lower middle Gravel (8)				
	Lower Gravel (8)				
	Lower Loam (8)				
Ingress Vale	Gravel (12)		0.298 (1)		0.30 ± 0.016 (10) [0.297 ± 0.009 (5)]
Little Thurrock	Brickearth (28)				
Clacton	Channel beds (12)			0.305 ± 0.001 (2)	0.299 ± 0.002 (3)
Sugworth	Organic beds (29)				0.296 ± 0.008 (4) [0.286 ± 0.016 (2)]
Nar Valley	Freshwater sands (12)	0.289 ± 0.026 (3)			
West Runton	Freshwater bed (30)			[0.346 ± 0.031 (5)]	0.348 ± 0.01 (9)
Little Oakley	Channel deposit (*)				0.344 ± 0.02 (6)
Purfleet	Shelly Gravel (12)		[0.38 ± 0.07 (5)]		
Yew Tree Farm	Freshwater beds (31)		0.33 ± 0.01 (3) [0.378 ± 0.08 (5)]		
Waverley Wood	Organic beds (7)				

The mean ratio and one standard deviation is given together with the number of shells analysed [parentheses]. The samples were analysed at the amino-acid geochronology laboratories at Aberystwyth; Royal Holloway and Bedford New College, London and, in parentheses, INSTAR, Boulder, Colorado. * R. C. Preece, unpublished data.

shown at Stutton, Essex, where fresh, unweathered Pleistocene fossils may be observed being incorporated into modern sediments.

The Standard Stages of Cromerian, Hoxnian and Ipswichian² are clearly separated by the extent of isoleucine epimerization in molluscs from each stratotype (Fig. 1, Table 2). In addition, however, the data show that other major geological events may also be identified in the stratigraphic record (Tables 1 and 2, Fig. 1). These are at Waverley Wood, Warwickshire⁷; Swanscombe, Kent⁸; and Stanton Harcourt, Oxfordshire⁹. The younger sites at Upton Warren, Worcestershire¹⁰, and Halling, Kent¹¹, also have characteristic amino-acid ratios (Table 2). These data are proposed as a new standard classification. Other stratigraphic units from localities throughout southern Britain are readily correlated with this aminostratigraphic framework (Table 1).

The aminostratigraphy (relative age) is calibrated by independent age determinations and thus converted into a geochronological scale. The Devensian late-glacial deposits at Halling, Kent, have a ¹⁴C date of 13 kyr BP¹¹. The Ipswichian is correlated with oxygen isotope substage 5e because the D/L ratios from the stratotype at Bobbitshole are similar to those at Bacon Hole Cave, Gower¹², where the stratigraphic unit containing *Cepaea nemoralis* has a uranium-series age of 122 kyr (ref. 13). The Bacon Hole marine beds have been previously correlated with the Eemian stratotype in the Netherlands⁵. Other thermoluminescence and U-series age determinations¹⁴, although less precise, also confirm this correlation. An electron-spin-resonance age determination on tooth enamel of 309 kyr from the lake deposits at Hoxne correlates the Hoxnian with oxygen isotope stage 9 (ref. 15). Using these geochronometric data as controls, the amino-acid ratios have been modelled against the global oxygen isotope signal as tuned to an orbital timescale^{16,17} (Fig. 1). This shows that evidence exists in Britain for correlation with all the odd-numbered stages of the oxygen isotope scale back to stage 15.

The wave-like form of the D/L ratios plotted against age (Fig. 1) is consistent with the kinetics of the epimerization reaction as shown by data from North American localities¹⁸. It is also consistent with the approximate position of the Matuyama-Brunhes magnetic reversal (730 kyr) used to calibrate aminostratigraphic studies in the Somme Valley of northern France (M. R. Bates, unpublished data).

TABLE 2 D-Alloisoleucine/L-isoleucine ratios from non-marine fossil molluscs from standard British stratotypes (bold), and data from other sites

Aberystwyth and London data	Boulder data
0.036 ± 0.001 (3) [Halling, Kent]	
0.066 ± 0.007 (5) [Upton Warren]	
0.09 ± 0.01 (6) Ipswichian	0.1 ± 0.005 (5)*
0.16 ± 0.016 (26) Stanton Harcourt	0.15 ± 0.006 (4)
0.26 ± 0.01 (4) Hoxnian	0.243 ± 0.023 (3)
0.3 ± 0.015 (14) Swanscombe and Ingress Vale	0.296 ± 0.005 (9)
0.348 ± 0.01 (9) Cromerian	0.346 ± 0.031 (5)
0.381 ± 0.024 (3) Waverley Wood	

The mean ratio and one standard deviation are given together with the number of shells analysed.

* From Trafalgar Square.

TABLE 3 Correlations of amino-acid geochronology with oxygen isotope stratigraphy

D/L ratio	British Isles	North-west Europe ²⁰	South-east France ¹	Δ ¹⁸ O Substage
0.066	Upton Warren Chelford	Odderade Brørup	St Germain II St Germain I	5a 5c
0.09	Ipswichian	Eemian	Eemian	5e

The Upton Warren event at several sites, correlated by their insect faunas, has been radiocarbon dated to between ~40 and 43 kyr (refs 10, 13). But the D/L ratios of 0.066 are incompatible with this because they appear to be closer in time to the Ipswichian (0.09), which is correlated with oxygen isotope substage 5e (~125 kyr). It is unlikely that only 0.02 of isoleucine epimerization occurred during ~85 kyr between the Ipswichian (125 kyr) and Upton Warren (~40 kyr), a time that included the two major interglaciations of substages 5c and 5a, while 0.03 of isoleucine epimerization occurred during ~27 kyr between 40 and 13 kyr when the climate was colder and large ice-sheets developed in Britain³. Thus, it seems reasonable to ascribe the Upton Warren sites to stage 5, probably substage 5a (~80 kyr), and the Upton Warren radiocarbon dates should be regarded as minimum ages. Table 3 shows important correlations between amino-acid geochronology and oxygen isotope stratigraphy.

By recognizing and dating additional events in the Pleistocene history of the British Isles amino-acid geochronology has allowed correlation with the oxygen isotope stratigraphy of the global ocean. It has also shown that existing models of glacial and environmental history, including Palaeolithic archaeology, are oversimplified, and require re-evaluation against this new standard for future challenges. The dating method is applicable to all continental sequences with non-marine fossil faunas. □

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Influence of coupling of sorption and photosynthetic processes on trace element cycles in natural waters

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CHEMICAL and biological processes have important roles in the transport and cycling of trace elements in natural waters¹⁻³, but their complex interactions are often not well understood. Trace-element concentrations may, for example, be controlled by adsorption-desorption reactions at mineral surfaces, with the equilibrium strongly influenced by pH. Variations in pH due to photosynthetic activity should result in concentration fluctuations as the adsorption-desorption equilibrium shifts with pH. To investigate these interactions, we have studied the effect of diurnal cycling of pH on dissolved arsenate in a perennial stream contaminated with arsenic. As expected, a diurnal cycle in arsenate concentration was observed, but surprisingly, the arsenate cycle lags several hours behind the pH cycle. Laboratory experiments show that the lag results from a slow approach to sorption equilibrium. Our observations demonstrate that the coupling of photosynthesis and sorption processes may have an important influence on the cycling of many trace elements and emphasize the importance of understanding sorption kinetics in modelling these processes.

Although diurnal pH cycles in surface waters are commonly reported^{4,5}, such cycles in the concentrations of trace elements have rarely been observed. Recent observations of diurnal fluctuations in iron and copper^{6,7} have been attributed to direct photochemical oxidation-reduction reactions, leading to concentration fluctuations that are correlated with light intensity. The concentrations of many trace elements are believed to be controlled by adsorption-desorption reactions at mineral surfaces^{8,9}, and pH is perhaps the most important of all variables that influence the extent of adsorption¹⁰⁻¹³. Thus, photosynthesis may have a significant, indirect effect on the concentrations of trace elements, because of the extreme sensitivity of adsorption-desorption equilibria to fluctuations in pH.

In Whitewood Creek, South Dakota, seepage of groundwater through contaminated, flood-plain deposits has caused an accumulation of arsenic-enriched iron oxyhydroxides in the stream bed and a general downstream increase in total arsenic abundance^{14,15}. Stream-water pH ranges from 8 to 9 and fluctuates by as much as 0.5 units daily due to photosynthesis, as evident from the coincidence of pH and light-intensity maxima (Fig. 1a) and diurnal variations of dissolved oxygen (Table 1). More than 98% of dissolved arsenic in the stream was present in the +5 oxidation state, referred to hereafter as arsenate. Diurnal cycles in the concentration of arsenate (C_{As}) were observed at two sites (Sheeler Seep, Fig. 1b; Custer, Fig. 1c), but these cycles lagged behind the pH cycles by several hours. At the Custer site a larger pH fluctuation was observed and the amplitude of the arsenate cycle was correspondingly greater (Fig. 1c). The general trend of C_{As} lagging pH fluctuations was observed at both sites for two consecutive days, but an accurate determination of the lag time was difficult because of the frequency of sampling and analytical uncertainties in C_{As} and pH.

The arsenate concentrations observed are orders of magnitude less than the solubilities of arsenic-bearing minerals which are stable in well oxygenated, neutral-pH environments^{16,17}. Control of C_{As} by adsorption on ferrihydrite is suggested by the strong affinity of arsenate for ferrihydrite surfaces¹⁸ and the constant As/Fe molar ratio (0.022 ± 0.006) observed in fresh precipitates

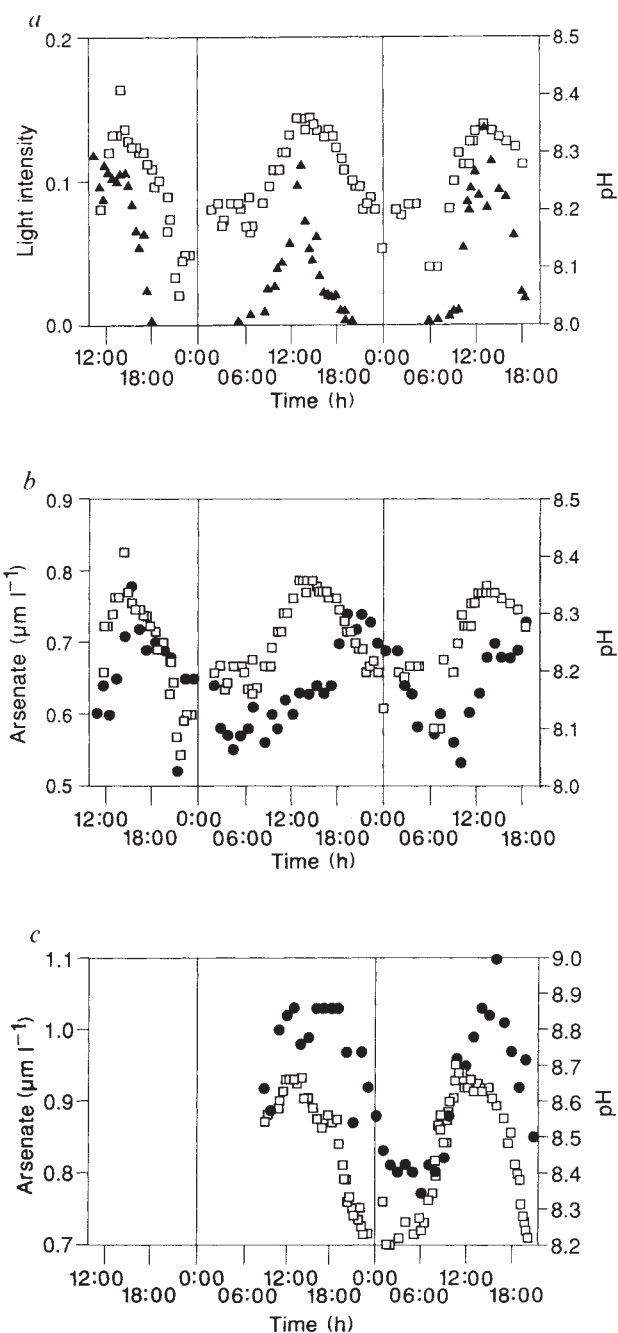


FIG. 1 a, Incident light intensity (▲) ($\text{einstein m}^{-2} \text{ min}^{-1}$) and stream-water pH (□) versus time of day. Data collected on 11–13 August 1987, at Sheeler Seep site, Whitewood Creek, 1.4 km upstream of confluence with Belle Fourche River. Site is characterized by extensive contaminated flood-plain deposits that discharge As- and Fe-bearing groundwater to the stream. Stream-water $[\text{SO}_4^{2-}] = 6 \text{ mM}$; total alkalinity 3 to 3.4 mequiv l^{-1} . Conservative tracer (LiBr) injected 2.8 km upstream of this site over 24-h period to quantify groundwater inflow and stream discharge. Light intensity (400–700 nm) measured at water surface. The pH electrode calibrated hourly with buffers at stream-water temperature. b, Dissolved arsenate (●) (micromolar) and pH (□) versus time of day, at Sheeler Seep site. Dissolved arsenic determined by hydride-generation atomic absorption spectroscopy on filtered (0.1 μm), acidified (1 ml l^{-1} HCl) water samples (average uncertainty in analyses $\pm 0.03 \mu\text{M}$). Preliminary analyses indicate >98% of dissolved As is As(v) determined by graphite furnace atomic absorption spectroscopy following separation by anion exchange²⁵. (Analytical methods discussed in detail in C.C.F. and J.A.D., manuscript in preparation). c, Dissolved arsenate (●) (micromolar) and stream-water pH (□) versus time of day. Data collected on 12–13 August 1987, at Custer site, Whitewood Creek, 10.4 km upstream of Sheeler Seep site. This site also in region of numerous visible groundwater seeps from contaminated flood-plain sediments.

and fine- and coarse-grain bed sediments¹⁹. To test this hypothesis, a series of laboratory experiments was conducted in which arsenate was co-precipitated with ferrihydrite in artificial stream water. Co-precipitation can occur by adsorption or inclusion of impurities in a precipitate²⁰. In the present context, co-precipitation means that arsenate is associated with the surface of ferrihydrite particles by means of adsorption, even though these surfaces may become 'internal surfaces' of coagulated colloids.

Arsenate adsorption decreased linearly as the pH increased from 8.0 to 9.0 in the co-precipitation experiments (Fig. 2). Sorption equilibrium was achieved within one hour after co-precipitation and C_{As} was constant for at least 24 h (Fig. 3). When the pH was increased by 0.5 units 24 h after co-precipitation, the approach to equilibrium was much slower (Fig. 3). Slow equilibration was also observed for re-adsorption of arsenate when the pH was decreased to its initial value (Fig. 3). The higher C_{As} attained at the end of the experiments was caused by slow transformation of ferrihydrite to more crystalline material (C.C.F. and J.A.D., manuscript in preparation). The initial changes of C_{As} in response to pH shifts resulted from adsorption-desorption of arsenate at ferrihydrite/water interfaces. Equilibrium was approached slowly, however, in comparison to the initial co-precipitation experiments, which was probably due to arsenate diffusion along surfaces within aggregates of coagulated ferrihydrite particles, as has been suggested for phosphate interactions with ferrihydrite²¹. It is clear from the above that adsorption reactions with ferrihydrite can yield arsenate concentrations close to those observed in Whitewood Creek. An accurate simulation of the field observations is difficult to achieve in the laboratory, however, because of numerous secondary factors, for example, precipitate age and incorporation of organic compounds.

The temporal concurrence of arsenate diurnal cycles at the two sites indicates that these cycles resulted from in-stream processes rather than physical mixing. Groundwater input of arsenic in a 2.8-km reach upstream from the Sheeler Seep site was estimated from an average groundwater arsenic concentration (3.8 μM) and an evaluation of groundwater inflow from a LiBr stream injection²². Accounting for removal during co-

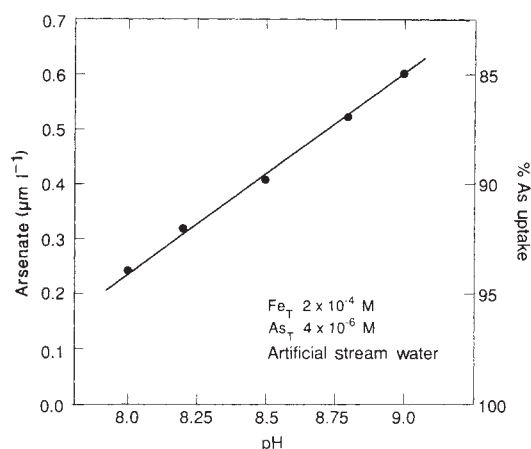


FIG. 2 Dissolved arsenate (micromolar) against pH, 24 h after precipitation of ferrihydrite in presence of arsenate. Experiments initiated by rapid, simultaneous additions of Fe^{2+} and C_{As} (labelled with ^{73}As) so as to maintain a constant As:Fe molar ratio and to yield total concentrations similar to those measured in groundwater (4 μM As and 200 μM Fe). Experiments conducted at 25 °C in artificial stream water of similar ionic composition and carbonate buffering capacity as Whitewood Creek, in equilibrium with the atmosphere. The pH was maintained (± 0.03 pH units) by acid and base additions with a pH controller. C_{As} determined by difference between total ^{73}As activity and solution activity (assayed by gamma spectrometry; $\pm 1\%$ based on counting statistics) of centrifuged subsamples (10,000g for 10 min).

precipitation with ferrihydrite (using laboratory data), a net change of only 0.03 μM arsenate would be expected from groundwater input during the pH cycle. An upper limit of 0.03 μM was estimated for daily arsenate removal by periphyton^{23,24} using the carbon fixation rate and molar As/C ratio measured in algae at the site (*Cladophora* and *Ulothrix*; J. Kuwabara, personal communication). The low concentration of Fe(II) observed ($< 0.1 \mu\text{M}$) suggests that the effect of iron photo-reduction⁶ on C_{As} was negligible.

None of the processes mentioned above can account for the 0.21 μM diurnal variation in C_{As} observed at the Sheeler Seep site (Fig. 1b). Although suspended sediment concentrations were too small to be significant, the bed sediments contained an abundant supply of adsorbed arsenate. We estimate that arsenate adsorption-desorption could cause a 4- μM change in C_{As} during the pH cycle, assuming that the stream water was in chemical equilibrium with only the top 1 cm of bed sediments. This calculation is based on the fraction of sorbed arsenate desorbed (1.5%) during a pH change from 8.15 to 8.4, determined from the results of arsenate sorption and isotopic exchange studies conducted with stream water and ferrihydrite precipitated from groundwater collected at the Sheeler Seep site (C.C.F. and J.A.D., manuscript in preparation). The average arsenic concentration measured in the 60–200- μm size fraction of bed sediments ($6.4 \pm 1.0 \mu\text{mol g}^{-1}$) from the site was used to estimate arsenate sorbed onto ferrihydrite coatings in the sediments. A contact area of stream water with bed sediment (273 l m^{-2}) was estimated from stream discharge, depth and width. Porosity of 0.5 and sediment dry density of 2.5 g cm^{-3} were assumed. The calculated 4- μM change is much greater than the observed change in C_{As} (0.21 μM), and could be due to a number of factors: the amplitude of the pH cycle within bed sediment may be smaller than in stream water; insufficient time to attain chemical equilibrium; the effect of iron oxyhydroxide ageing on arsenate desorption; depth of stream-water/pore-water mixing within bed sediments.

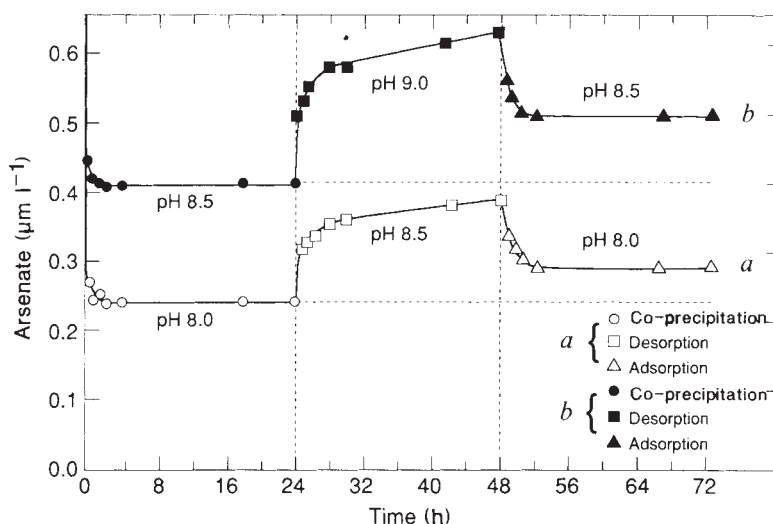
From these estimates and the laboratory observations, it is apparent that adsorption-desorption processes in bed sediments were largely responsible for the diurnal arsenate cycle and the control of C_{As} . At the Custer site, C_{As} was greater due to a higher pH and a slightly larger As:Fe molar ratio in the bed

TABLE 1 Stream-water dissolved oxygen and pH, 4 km upstream of Sheeler Seep site, Whitewood Creek

Date	Time (h)	Dissolved oxygen (μM)	pH
7 July	15:00	347	8.49
	16:00	344	8.52
	17:00	306	8.50
	18:00	200	8.40
	19:00	166	8.30
	20:00	153	8.21
	21:00	144	8.14
	22:00	138	8.10
	23:00	153	8.06
	24:00	166	8.02
8 July	01:00	175	8.03
	02:00	175	8.06
	03:00	181	8.04
	04:00	181	8.04
	05:00	184	8.04
	06:00	194	8.06
	07:00	219	8.10
	08:00	250	8.18
	09:00	294	8.25
	10:00	328	8.34
	11:00	347	8.39
	12:00	369	8.43
	13:00	369	8.46

Data collected 7–8 July, 1987. Dissolved oxygen determined by oxygen electrode.

FIG. 3 Dissolved arsenate (micromolar) against time, following co-precipitation with ferrihydrite (a, pH 8.0 to 24 h; b, pH 8.5 to 24 h). pH increased by 0.5 units after 24 h of reaction by rapid base addition; pH decreased by 0.5 units after 48 h by rapid acid addition. Total As, 4 μM ; total Fe, 200 μM .



sediments, which is consistent with arsenate adsorption isotherms¹⁸. These results are significant, because few studies have conclusively demonstrated that sorption processes constrain the concentration of a trace element in a natural system.

The observations of arsenate fluctuations have important implications for strategies used in the sampling of surface waters and the use of trace-element concentrations in geochemical or limnological models. Quantitative geochemical models that predict natural water composition are frequently based on chemical equilibria between water and solid phases. But the transient fluctuations in pH caused by photosynthesis can lead to significant fluctuations in trace-element concentrations and contribute to a general state of disequilibrium with respect to sorption reactions. Fluctuations in pH in surface waters more acidic than Whitewood Creek might be expected to have a greater effect on trace cations, for example, Zn, Cu and Cd, because these ions are very strongly adsorbed in alkaline waters^{12,13}. It has been argued⁶ that diurnal fluctuations of iron may contribute to the greater abundance of amorphous iron hydroxides over that of crystalline forms in streams. Similarly, a continual disturbance of sorption equilibria may increase the internal cycling of many trace elements within aqueous systems and may significantly affect the fate, transport and bioavailability of phosphate, micronutrients and contaminants. A full understanding of natural systems will require that we understand the complex ways in which seemingly unrelated processes such as photosynthesis and sorption are coupled. □

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Global coupling of Earth surface topography with hotspots, geoid and mantle heterogeneities

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REMARKABLE global correlations exist between the Earth's gravity field (the geoid), hotspot distribution and lower-mantle heterogeneities. The geoid has two conspicuous long-wavelength highs centred on the Equator 180° apart ('degree 2 pattern'), which contain most of the world's hotspots¹⁻⁴. Seismic tomography of the lower mantle has revealed that low-velocity, presumably hot, regions are positively correlated with hotspot concentration and geoid highs⁴⁻⁷. In a viscous, convective Earth, correspondence between geoid highs and hot (low-density) mantle implies dynamical uplift of mantle boundaries, particularly the Earth's surface^{8,9}. The gravitational effect of the surface deformation caused by the convective flow is opposite in sign and comparable in magnitude to that arising from the deep density contrast; geoid anomalies result from these mutually opposing contributions in a manner that depends critically on the dynamics of the mantle, that is, on viscosity stratification and style of convection. Here we show that the global surface topography, once corrected for shallow density variations inside the lithosphere, presents a strong degree 2 pattern, with uplifted regions well correlated with hotspot concentration, geoid highs and hot lower mantle. This long-wavelength topography probably represents the dynamic response of the Earth's surface to mantle convection; used together with geoid and seismic velocity anomalies, it should provide an important constraint on convection models.

Recently, seismic tomography has revealed long-wavelength

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velocity anomalies in the mantle^{6,10}, which have been interpreted in terms of geoid anomalies on the basis of dynamical circulation models^{7,11}. Although it is known on theoretical grounds that the dynamical surface topography is the dominant density contrast contributing to the observed geoid, and although this quantity is predicted by circulation models^{7,11}, observational investigation has not yet confirmed it. The Earth's topography is dominated by shallow density contrasts within the lithosphere resulting from variations in crustal thickness of continents, especially at convergence zones, and from cooling of the oceanic lithosphere. However, these effects give a negligible contribution to the long-wavelength geoid because of isostatic compensation. To isolate the dynamical response of the Earth's surface to internal convective forces, it is necessary to remove the topographic components resulting from near-surface density contrasts, which are responsible for the baseline difference between continents and oceans. For ocean bathymetry, these corrections include sea-floor subsidence with increasing crustal age, caused by cooling of the oceanic lithosphere, and sea-floor uplift by the load of isostatically compensated sediments. As the bathymetry is referred to sea level, the mean depth of mid-ocean ridges is also subtracted. Corrected bathymetry measurements reveal positive and negative topography anomalies. For the continental topography, corrections are less straightforward. Ideally, one should correct first for variations in crustal thickness and then remove the mean isostatic elevation of the residual topography. In practice, the first correction at present remains subject to large uncertainties. We prefer to adopt a simple procedure in which we exclude major convergence zones (orogenic belts and plateaus) which are essentially associated with deep crust. Then we subtract the mean elevation of continents (convergence zones and ice sheets excluded) to ensure that the global residual topography has zero mean value, thus avoiding any offset between continents and oceans. We will see below that the influence of continental corrections is in fact minor and that, more generally, continents contribute little to the residual long-wavelength topography. Richards and Hager⁵ found no correlation between major continental reliefs and the long-wavelength geoid.

The topographic data source used in this study is the ETOP05 database available from the National Oceanographic and Atmospheric Administration in Boulder, Colorado. This provides worldwide digital land and sea-floor elevations on a $5' \times 5'$ grid. According to simple thermal models of conductive cooling of oceanic plates, sea-floor subsidence B and mean depth A of mid-ocean ridges are taken into account through a function of the type $A + B \times (\text{age})^{1/2}$. Several age-depth relationships based on worldwide bathymetry analyses have been proposed (see, for example, refs 12, 13). Here we consider the following relationships: (1) $2,500 + 350 \times (\text{age})^{1/2}$ for crustal ages less than 70 Myr, $6,400 - 3,200 \exp(-\text{age}/63)$ for crustal ages greater than 70 Myr (ref. 12); (2) $2,700 + 300 \times (\text{age})^{1/2}$ (ref. 13); and (3) $2,400 + 315 \times (\text{age})^{1/2}$ (J. C. Marty and A. Cazenave, preprint). The above values of the parameters A and B are in m and $\text{m Myr}^{-1/2}$ respectively, with age in Myr. It is worth noting that the corrected bathymetry based on relationship (3) has a mean value of nearly zero. Isostatically compensated sediments of thickness S produce an uplift of the ocean floor of the form $(\rho_m - \rho_s)/(\rho_m - \rho_w)S$, where ρ_m , ρ_s and ρ_w are mantle, sediments and sea-water density respectively. This uplift is added to the observed sea-floor depth using digitized sediment-thickness data (see refs 14 and 15 for details of these corrections). The corrected topographic map over the world oceans, based on the age-depth relationship given in ref. 12, is presented in ref. 15. It is dominated by two large-scale topographic highs over the western Pacific and around Africa, suggestive of a degree 2 and order 2 pattern. For the continental areas, we remove reliefs associated with active tectonic processes, such as mountain ranges and plateaus, by excluding data from elevations above 1,000 m; this procedure eliminates most orogenic belts and large plateaus

such as Tibet that result from surface tectonics. Using a higher cutoff of 2,000 m results in negligible differences. Ice sheets (Greenland and Antarctica) and continental margins have not been considered.

As is standard practice for surface fields, the global corrected topography is represented by a spherical-harmonic expansion in order to compute its power spectrum (or degree variance), its degree correlation (spatial coherence) with other global fields (geoid, hotspot distribution and seismic velocity anomalies at different depths in the mantle) and the transfer function (admittance) with the geoid. Theoretical expressions for these quantities are given in ref. 14. Spherical-harmonic coefficients are computed by applying an integration technique.

The power spectrum of the global corrected topography has an energy maximum at degrees 2 and 3. Corresponding degree variances are ~ 150 m regardless of the corrections applied. We will not discuss further the degree 3 peak as it shows no correlation with other global fields. The degree 2 pattern of the corrected topography is shown in Fig. 1. Three different formulations of this degree 2 pattern are presented: for (1) corrected topography over oceans (based on the age-depth relationship of Marty and Cazenave (preprint)), and continental topography (convergence zones, ice sheets and margins excluded) corrected for the mean residual elevation (Fig. 1a); (2) corrected topography over oceans only (subsidence correction as in (1)), with all continental areas excluded (Fig. 1b); and (3) corrected topography over

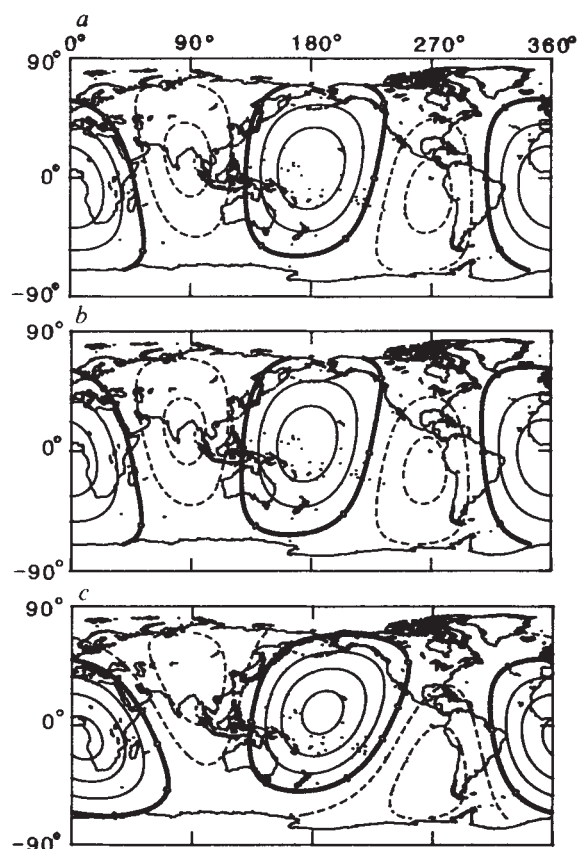


FIG. 1 Degree 2 pattern of the spherical-harmonic expansion of the surface topography. *a*, Oceanic areas are corrected for sea-floor subsidence (expressed as $2,400 + 315 \times (\text{age})^{1/2}$ metres) and sediment loading. For continental areas, convergence zones, ice sheets and margins are excluded and mean residual elevation of continents is removed. *b*, Oceanic areas only, with corrections made as in *a*. *c*, Same as *a* for continental corrections, but using the age-depth relationship for sea-floor subsidence given in ref. 12. In all figures, solid (dashed) curves represent positive (negative) topography. Contours are at 100-m intervals.

oceans (based on the age–depth relationship given in ref. 12), and continental topography as in (1) (Fig. 1c). The degree 2 patterns presented in Fig. 1a–c are similar. In Fig. 1a and b the condition of zero mean for the corrected topography is satisfied. Several other cases have been considered, to investigate the effects of continental corrections and of applying the age–depth correction given in ref. 13. All results present a fairly stable degree 2 topography dominated by a tesseral (order 2) figure. The degree 2 topography is characterized by two broad antipodal highs, ~300 m in amplitude, centred at the Equator over western Africa and the western Pacific. The degree 2 topography closely resembles the degree 2 pattern of hotspot distribution, geoid and lower-mantle velocity anomalies reported by Richards and Hager^{4,5}.

We have quantified the correlation between corrected surface topography and other global fields (hotspot distribution, geoid and seismic velocities) using the spherical-harmonic expansion of each field. We have used the 47 hotspots listed in ref. 4. Geoid data are based on the recent geopotential model GEMT1¹⁶. We refer the geoid to the geometric (rather than hydrostatic) ellipsoid because the long-wavelength topography is clearly dominated by a degree 2 and order 2 figure and shows no evidence of flattening. Seismic velocity anomalies are obtained from the model M84C for the upper mantle¹⁰ and L0256 for the lower mantle⁶. The power spectrum of the hotspot distribution peaks at degrees 1, 2 and 6. Maps of the degree 2 and degree 6 hotspot distribution are presented in ref. 4. We have calculated the correlation coefficient r between hotspot distribution and corrected topography, for each of the different cases discussed above. In all cases, maxima are observed at degree 2 ($r \sim 0.9$) and degree 6, suggesting a causal relationship between the two surface fields at corresponding wavelengths. We have established that the long-wavelength topography cannot be the consequence of topographic hotspot swells: using the results of two recent studies (ref. 17 and M. Monnerneau and A. Cazenave, preprint), in which the observed spatial extent (1,000–1,500 km) and height (500–1,500 m) of hotspot swells were determined, we have calculated the spherical-harmonic expansion of the swell topography. Around each of the 47 hotspots, observed swell height and width are determined assuming a gaussian shape for the swell. The power spectrum of the swell topography

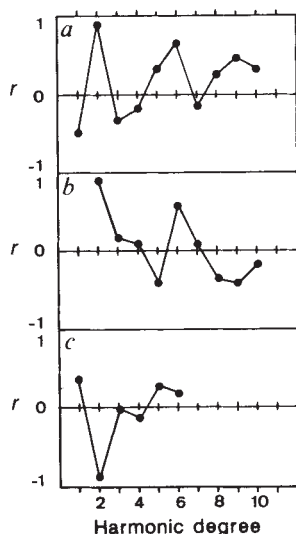


FIG. 2 Correlation as a function of harmonic degree between corrected global surface topography (as defined for Fig. 1a) and a, hotspot distribution, b, geoid, and c, lower-mantle velocity anomalies at 2,500 km depth (negative correlation indicates that positive topography corresponds to low-seismic-velocity regions).

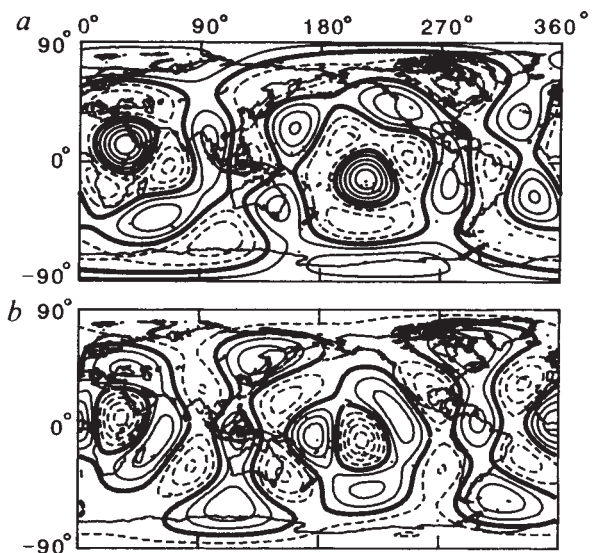


FIG. 3 Degree 6 pattern of the spherical-harmonic expansion of a, topography of hotspot swells (contours at 20-m intervals; solid (dashed) curves represent positive (negative) topography); and b, upper-mantle velocity anomalies at 400 km (contours at 0.01-km s⁻¹ intervals; solid (dashed) curves represent high (low) velocities).

resembles that of hotspots, with peaks at degree 1, 2 and 6. The corresponding degree variances are 25 m, 30 m and 31 m respectively. The correlation with the long-wavelength topography is peaked at degree 2 and 6, as is the hotspot distribution. However, the swell topography represents less than 20% of the observed long-wavelength topography. We conclude that a dynamic component of degree 2, of amplitude ~250 m, probably of convective origin, still remains. Note that the observed dynamic topography is smaller by a factor of three than that predicted theoretically^{7,11}. We have calculated the correlation between corrected topography and the geoid. The geoid has not been corrected for effects of subducting slabs because topography may eventually contain a slab component. As with hotspots, correlation peaks are observed at degree 2 ($r = 0.9$) and degree 6 for all cases of corrected topography. Figure 2 shows the correlation between corrected topography and hotspot distribution (Fig. 2a) or geoid (Fig. 2b) as a function of harmonic degree. The computed transfer function between geoid and topography is ~150 m km⁻¹ at degree 2 and ~25 m km⁻¹ at degree 6. The degree 2 admittance is suggestive of deep compensation of the topography, as expected if the topography is related to global mantle circulation, which is suggested by the high correlation between surface topography and low seismic velocities in the lower mantle (Fig. 2c). The lower admittance at degree 6 probably reflects compensation in the upper mantle; using an averaged upper-mantle density, we have deduced an apparent depth of compensation of ~400 ± 50 km for the degree 6 topography. We believe that the two correlation peaks at degree 2 and 6 observed between topography, hotspots, geoid and mantle heterogeneities reflect characteristic modes of convection. A degree 2 mode in the lower mantle inducing a degree 6 mode in the upper mantle. This is consistent with the suggestion⁴ that hotspot plumes form in hot mantle regions. We find correlation peaks between hotspot concentration and low seismic velocities at degree 2 for lower-mantle velocities and at degree 6 for upper-mantle velocities. Incidentally, this study relates the Afar–African Rift region and the Polynesian province, known for their high hotspot density, to the degree 6 of hotspot distribution and swell topography (Fig. 3a). Figure 3a presents a characteristic pattern with two antipodal uplifted regions, Afar and Polynesia. Seismic velocities of between 350 km and 500 km in the upper mantle

show a very similar pattern at degree 6 (Fig. 3b). The Polynesian province (the 'south Pacific superswell'¹⁸) has recently been recognized to be anomalous in many respects¹⁹. Along with the Afar-African Rift region, it corresponds to a region of hot upper mantle and is probably related to global plume dynamics. □

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Climate and signature years in west European oaks

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It has been shown that in certain 'signature' years tree-ring chronologies from living oaks throughout much of western Europe exhibit the same characteristic of either increasing or decreasing growth¹. Remarkably, these chronologies, many of which are the product of the recent extension of the European tree-ring network²⁻⁴, are distributed over a latitude span of ~10° and a longitude span of 20°. This phenomenon means that archaeological series from more distant areas can be compared, increasing the likelihood that gaps in regional dating chronologies can be bridged^{5,6}. The question arises as to what circumstances could induce trees over such a large area to behave in unison so frequently. We have examined climate departures from modern signature years to determine whether or not there is a typical climate pattern associated with years of large-scale growth increase or decrease. We find that particular climatic conditions affecting the area from which the tree-ring chronologies are drawn can account for the signature years and suggest that this relationship may enable archaeological chronologies to be used in climate reconstruction.

In cross-dating oaks, growth patterns for timbers or trees from one source area are compared and consolidated into an undated site chronology. They are then fixed in time by reference to living trees or to chronologies from other sites. The simplest comparison that may be carried out between these time series is based on the signs of the first differences: if the ring in a particular year is wider than the ring of the previous year then the sign will be positive whereas if it is smaller, the sign will be negative. Chronologies from the same site or from sites in related areas will have similar patterns of signs. The degree of this concurrence has been used extensively as a measure of the

similarity of tree-ring chronologies; the appropriate statistic is known as the coefficient of parallel variation or 'Gleichläufigkeitswert'⁷.

Signature years, which for the purpose of this analysis are defined as years in which 32 (80%) or more of the chronologies show the same sign of the first difference (Table 1), were identified in a set of 39 chronologies (Fig. 1) covering the period 1851-1970. Out of the total of 120 years, 10 positive signature years (years in which a general increase in growth occurred) and 10 negative signature years were identified (Table 2). There were two relatively long periods when no signature years occurred: 1851-1867 and 1925-1949.

Three data sets were used to define the climatic character of the signature years: gridpoint mean-sea-level pressure data (provided by the UK Meteorological Office)⁸, surface-air and sea-surface temperature data⁹, and precipitation data^{10,11}. The reliability of these data is good over the North Atlantic-European study area although the coverage is reduced during the nineteenth century and the quality of the data is poorer during the early years of the records. The precipitation data are restricted to land and the marine temperature data are prone to long-term biases owing to changes in instrumentation, observational technique, and so on.

A simple compositing technique was used to isolate any climatic signal that may exist in the sets of signature years. Gridpoint data for the two samples of signature years were averaged to determine whether or not there was any consistency in the character of the two sets of years. The resulting composites, in the form of departures from a reference period mean, were tested for significance gridpoint by gridpoint using the *t* test. The compositing was performed on data for the 6-month season, March to August, and the spring, March to May, and summer, June to August, periods of the signature year.

The chart of pressure departure at mean sea level for the March-August season of the positive signature years shows a

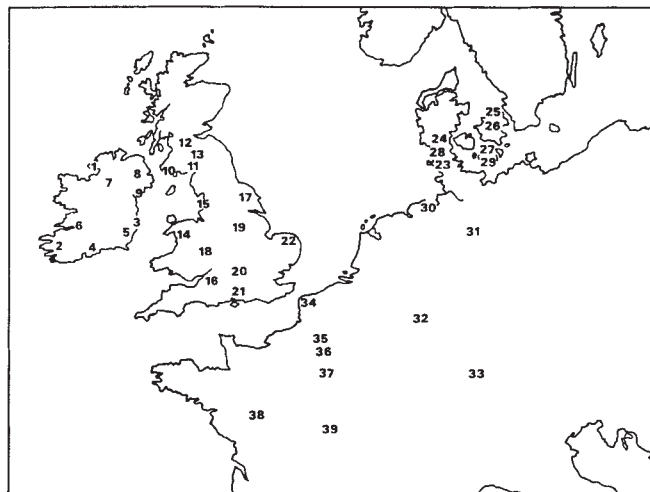


FIG. 1 The oak chronology network: 1, Ardara¹³; 2, Killarney¹³; 3, Glen of the Downs¹³; 4, Cappoquin¹³; 5, Eniscorthy¹³; 6, Lough Doon¹³; 7, Castle Coole⁴; 8, Shanes Castle⁴; 9, Rostrevor¹⁴; 10, Glenluce¹⁵; 11, Raehills¹⁵; 12, Cadzow⁴; 13, Lockwood¹⁵; 14, Maentwrog¹⁶; 15, Scorton¹⁵; 16, Bath¹⁵; 17, York, R. Morgan (personal communication); 18, Ludlow¹⁵; 19, Sherwood⁴; 20, Oxford¹⁵; 21, Winchester¹⁷; 22, Blickling¹⁵; 23, Draved⁴; 24, Gram⁴; 25, Jægerspris, M. K. Hughes & D. M. Brown; 26, Kong Frederik den Vs Allé, M. K. Hughes & D. M. Brown; 27, Kristiansæde, M. K. Hughes & D. M. Brown; 28, Lindet⁴; 29, Pederstrup⁴; 30, Niedersächsen¹⁸; 31, Göttingen¹⁹; 32, Hollstein²⁰; 33, A100, B. Becker (personal communication); 34, Foret de Guines, J. R. Pilcher (personal communication); 35, Oise²¹; 36, Halatte, J. R. Pilcher (personal communication); 37, Fontainebleau⁴; 38, Chinon, J. R. Pilcher (personal communication); 39, Tronçais⁴.

TABLE 1 A sample set of years with signature years highlighted

Year	Chronology																																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	
1940	-	-	+	-	-	+	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	+	
	+	+	+	+	+	-	-	+	+	+	-	-	+	+	-	-	-	-	-	-	=	-	+	=	+	+	+	-	+	+	+	-	-	+	+	=	+	-	-	-
	-	+	+	+	+	+	-	-	+	-	-	-	-	+	-	+	-	=	+	-	+	+	+	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	-
	-	+	-	-	+	-	-	-	-	-	=	-	+	-	+	+	-	+	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	-
	-	-	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	=	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	=	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+
	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	=	-	-	-	-	+	=	-	-	+	+	+	+	-	=	+	+	+	+	
	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	-	
	-	-	-	+	-	+	-	-	-	-	-	+	=	+	-	+	+	=	-	+	-	+	-	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	+
1950	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	↑*	
	-	+	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	=	+	+	
	-	-	-	-	-	+	-	-	-	=	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-		
	+	+	+	+	-	-	+	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	-	+	+	+	-	-	=	-	+	+	-	-	=	-	+	+		
	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	↓		
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	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	-		
	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	↑		
	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	↑		
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Signature years are defined as years in which 80% or more of chronologies exhibit a first difference of the same sign. We used a Monte Carlo simulation technique to assess the statistical significance of the observed frequency of signature years, as an assessment based on the binomial distribution would give a spuriously high significance through ignoring autocorrelation effects. We selected a 120-yr portion of the set of chronologies and realigned the individual chronologies by giving them random start dates, then counted the number of signature years in the set of realigned chronologies. Repeating this procedure 2,000 times showed that both positive and negative signature years occurred at a frequency of only 1 in every 10,000 years by chance.

+ Growth increases from the previous year.
- Growth decreases from the previous year.
= No change from the previous year.
↓ Negative signature year.
↑ Positive signature year.
* 90% or more of the chronologies have a first difference of the same sign.

strong, spatially coherent pattern (Fig. 2a). Pressure is lower than normal over western Europe with a negative anomaly-centre near the United Kingdom. Positive pressure anomalies dominate the north of the study area. Clearly, during years in which growth increases there is a tendency for cyclonic activity to be enhanced over middle latitudes of western Europe. As might be expected, this is accompanied by an increase in precipitation (Fig. 2b, c). There is evidence for a positive response of oak growth to enhanced precipitation in this area that would explain the tendency to more rapid growth throughout the network in these years¹². Although temperatures are slightly below normal over the tree-ring network during these years, there are no notable anomalies; we conclude that temperature does not play a main role in producing these signature years.

The pressure-departure pattern for the years with decreasing growth is presented in Fig. 2d. The only significant feature near the region of the tree-ring network is the area of positive pressure anomalies over the eastern Mediterranean. The precipitation composites show no notable departures in the region of the network. The temperature composite for the March–August season (not shown), however, reveals a strong pattern: cooling in the northeast of the network and warmth to the southwest. This broad pattern appears in both the spring and summer composites although the anomalies over the northeast of the network are most prominent in spring (Fig. 2e) whereas the warmth in the southwest of the network is a summer feature

(Fig. 2f). These temperature departures are consistent with the change in atmospheric flow and synoptic activity suggested by the pressure composite. The cause of the large-scale decrease in growth seems to be the northeast/southwest difference in the

TABLE 2 The signature years used in the compositing

Increasing	Decreasing
1871	1868
1875	1876
1890	1887
1903	1893
1910	1898
1917	1905
1924	1919
1950	1923
1958	1956
1960	1970

The pressure record begins in 1873 and earlier years were not used in the pressure composites. To test the reproducibility of the results of the compositing, these samples of signature years were split into two subsets (based on alternate years in the two samples) and composites were produced for these four sets of years. In the case of the temperature analyses, the samples were also split into subsets based on the early and later halves of the record in order to test for long-term bias in the marine data. Any feature noted in the discussion was present in each of the subsample composites.

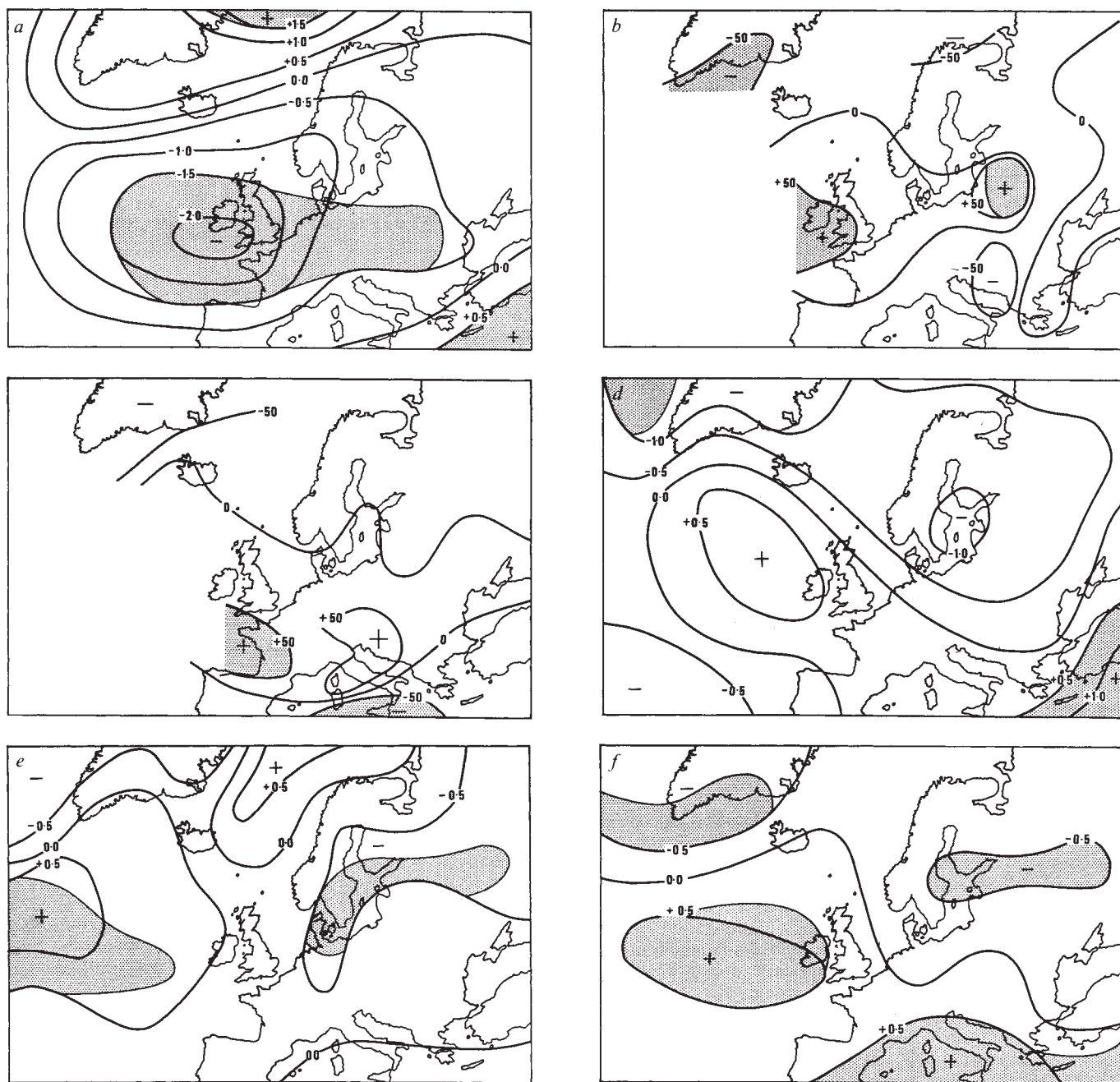


FIG. 2 Climatic departures associated with the positive signature years. *a*, Departure of mean-sea-level pressure (mb) from the 1951-80 mean: March-August. *b*, Precipitation departure (mm) from the 1951-80 mean: March-May. *c*, Precipitation departure (mm) from the 1951-80 mean: June-August. Climatic departures associated with the negative signature years. *d*, Departure of mean-sea-level pressure (mb) from the 1951-80 mean: March-August. *e*, Departure of surface air temperature ($^{\circ}\text{C}$) from the 1951-80 mean: March-May. *f*, Departure of surface air temperature ($^{\circ}\text{C}$) from the 1951-80 mean: June-August. Shading indicates significance at the 5% level.

character of the temperature anomalies over the region of the network. Briffa¹² found that cool summers had a negative effect on ring-width in northern Britain and Ireland but a positive effect in southern England and northern France.

We have identified recurrent patterns of climatic conditions associated with large-scale signature years in modern European oak tree-ring chronologies. This suggests that climatic information could be derived from archaeological chronologies, which was previously considered problematic as the trees contributing to archaeological chronologies could not be accurately provenanced. The existence of signature years in which growth patterns are related over a wide area means that information concerning

provenance is not crucial. There are periods during the last millennium for which large quantities of dated oaks are available across western Europe and the varying frequency of occurrence of the signature years may provide a useful source of climate information. □

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Fine-scale adaptation of herbivorous thrips to individual host plants

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SHORT-LIVED sedentary herbivores that feed on long-lived plants can complete hundreds or thousands of generations on a single host genotype. Insects are known to adapt rapidly to changes in the resistance characteristics of their host plants¹ and new plant cultivars bred for resistance to specific pests 'lose' their resistance within a few years due to evolution of the pests. These observations led to the proposal that natural populations of scale insects become differentiated into small demes, each specialized on an individual pine tree². This hypothesis predicts that a herbivore found on and hence adapted to, host individual A should have a higher fitness than should insects from other unrelated conspecific host individuals, when both are reared on host A. Testing this hypothesis by measuring the population growth of thrips (*Apterorhrips secticornis* Trybom, Thysanoptera: Thripidae) on individual clones of their host (*Erigeron glaucus* Ker., Compositae), I found that their growth was greater on cuttings of their native plant clone than on other clones. This was not the result of environmental conditions experienced by insects or their hosts, but rather, of genetic adaptation and is the first demonstration of fine-scale adaptation of a herbivore to individual clones of its host plant.

E. glaucus forms dense mats of many rosettes connected near ground level³. Clones are at least a decade and possibly hundreds of years old⁴. *A. secticornis* is a specialist on *E. glaucus* at the study site of Bodega Bay in Sonoma County, California. Thrips are active at Bodega Bay throughout the season, completing a generation in 2–8 weeks depending upon temperature⁴. These

TABLE 1 Results of ANOVA of number of thrips per plant in the common garden

Source	First Generation			Third Generation		
	d.f.	F	P	d.f.	F	P
Plant clone	2	0.03	0.97	2	0.10	0.90
Thrips line	2	1.21	0.30	2	0.26	0.77
Plant clone × Thrips line	4	1.93	0.11	4	4.82	0.002
Error	81			81		

thrips are wingless and sedentary. Dispersal among plant clones is uncommon although this has not been quantified in the field. They reproduce parthenogenetically although some sexual reproduction probably occurs, as has been reported for a congeneric species⁵. I selected three clones of *E. glaucus* growing within 500 m of each other such that I included the total range of thrips density observed at the study site; in monthly censuses one clone consistently supported 0–0.2 thrips per rosette, another supported 2–20 per rosette, and a third was intermediate⁴.

To examine whether thrips had adapted to their host clones, I compared their population growth on cuttings of different origins. Rosettes of *E. glaucus* were cut and rooted during the rainy season of 1985–86. I randomly interspersed 30 rosettes of each of the three plant clones in a common garden. Plants in this garden were kept free of thrips by frequent hand removal from October 1987 to January 1988. The thrips used in the experiments were from lines taken from the three clones and reared on a single new plant genotype. The lines or colonies of thrips were started from 25 adults from each of the three clones and they were maintained in the greenhouse on a common clone which was not one of the three experimental clones. After two complete generations of rearing on plants of this common clone, thrips were transferred back to the cuttings in the garden on 12 January 1988. By rearing all thrips for two generations on a single new plant genotype, I ensured that any potential conditioning effects were the same for all thrips lines. All leaf tissue fed upon by thrips in this experiment (spring 1988) had been produced after plants had been transferred to the common garden (December 1985). By allowing the cuttings to grow for 2 years in the common garden before initiating the experiment, I minimized conditioning effects on the plants. Three immature thrips were placed on each plant in the common garden such that each combination of plant clone and thrips line was replicated 10 times. Placement of thrips on rosettes was determined by random numbers such that 10 plants of clone 1 received thrips from line (i), 10 received thrips from line (ii), 10 received thrips from line (iii); similarly 10 plants from clones 2 and 3 received thrips from each of the three lines. Each rosette was enclosed in a cage made of white organdy to prevent dispersal of thrips and reduce predation³. Thrips populations were counted biweekly and subjected to statistical analysis after one generation and again after three generations.

After three generations, neither plant clone nor thrips line significantly affected the number of thrips per plant (Table 1).

TABLE 2 Number of thrips per plant in the common garden

Plant clone	(i)	Thrips line			Third generation	
		First generation	(iii)	(i)	(ii)	(iii)
1	3.0 ± 0.8	1.9 ± 1.3	1.9 ± 0.9	22.9 ± 7.7	5.8 ± 2.2	7.9 ± 2.3
2	1.3 ± 0.4	3.3 ± 0.9	2.7 ± 0.7	7.8 ± 1.6	17.8 ± 5.4	11.8 ± 4.0
3	1.2 ± 0.6	1.4 ± 0.4	4.7 ± 2.0	8.2 ± 2.3	10.8 ± 2.6	21.9 ± 4.6

Mean ± standard error. Thrips line (i) was taken from plant clone 1, line (ii) from clone 2, line (iii) from clone 3.

The interaction between these two variables, however, was significant, indicating that thrips population growth depended on the particular plant clone on which it was found. Any deviation from the marginal probabilities will cause the interaction term to be significant; therefore a significant interaction is not sufficient evidence for the deme formation hypothesis^{6,7}. In both the first and third generation, however, the significant interaction was that predicted *a priori* by the hypothesis of local adaptation: thrips increased more rapidly on their plant clone of origin than on other clones (Table 2). This specific hypothesis was tested by *a priori* contrasts⁸. For the first generation: $F_{1,81} = 7.12$, $P < 0.01$; for the third generation: $F_{1,81} = 17.70$, $P < 0.01$.

Previous workers have looked for evidence of adaptation of herbivorous insects to their host plant genet. Edmunds and Alstad conducted transfer experiments of scales within a single host (intratree transfers) and transfers between the original host and other conspecifics located several kilometres away (intertree transfers)². They found higher survival for scales in intratree transfers compared to intertree transfers, which is consistent with but does not prove the deme formation hypothesis. Because all of the intertree transfers involved a set of trees located at a distant site, however, it was impossible to separate the hypothesis of adaptation to particular trees from the alternative of adaptation to particular sites⁹. In addition, Edmunds and Alstad did not establish whether the differential survival of scales on different trees was the result of genetic adaptation and not physiological acclimation^{9,10}. Similarly, in several other studies reporting interactions between plant genets and insect lines replicated arrays of plant propagules in a common environment were not used^{11,12}. As such, the results could have been caused by undetected environmental differences between plants rather than by genetic differences. Studies that have tested the deme formation hypothesis rigorously have failed to find convincing evidence to support it^{7,13,14}.

These are the first results indicating that a herbivore has adapted to individual plant clones. Because the plant cuttings and the colonies of thrips were raised in a common environment, it is very unlikely that these results were caused by behavioural or physiological conditioning^{15,16}. Insects should show local adaptive divergence if selection is strongly dependent on the host plant, if selection remains directional for many generations and if gene flow is reduced between hosts. This study suggests that selection does vary among neighbouring conspecific plants. And because thrips may complete hundreds of generations on a plant, selection will probably be consistent over time. Winglessness and asexual reproduction probably reduce gene flow although movement in the field has not been quantified. Despite some gene flow, differential growth has produced a thrips population structured by its host plants. □

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Very high frequency of lymphoma induction by a chemical carcinogen in *pim-1* transgenic mice

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INFECTION of mice with Moloney murine leukaemia virus (MuLV) induces T-cell lymphomas after an average latency period of 150 days. In these lymphomas the MuLV DNA is frequently integrated into the mouse chromosomal DNA in the vicinity of the *pim-1* oncogene¹. Transgenic mice overexpressing the *pim-1* oncogene are predisposed to develop T-cell lymphomas, but only to the extent that ~10% of the mice develop a lymphoma within 240 days. When these mice are infected with MuLV, lymphomas develop in all mice in only 50–60 days². In these lymphomas MuLV DNA is integrated near either the *c-myc* or *N-myc* gene, suggesting that *pim-1* and *myc* synergize in lymphomagenesis^{1,2}. To determine whether this system has a more general application, we have now tested the susceptibility of *pim-1* transgenic mice to *N*-ethyl-*N*-nitrosourea (ENU), a chemical carcinogen. With a single low dose of ENU, nearly all *pim-1* transgenic mice, but only 15% of non-transgenic mice, develop T-cell lymphomas within 200 days. All ENU-induced lymphomas in both *pim-1* transgenic and non-transgenic mice express high levels of *c-myc* messenger RNA, supporting the notion that *pim-1* and *c-myc* synergize in lymphoma induction². We propose that *pim-1* transgenic mice could be used to test the oncogenic potential of other chemical compounds.

We used two different *pim-1* transgenic mouse lines: *Eμ-pim-1* and *H₂K-pim-1* mice; the constructs generating these mice are shown in Fig. 1. Both the *Eμ-pim-1* and the *H₂K-pim-1* transgene are expressed predominantly in lymphoid cells² (J. Domen, personal communication). Within 240 days, 10% of the *Eμ-pim-1* mice develop spontaneous T-cell lymphomas, whereas none of the *H₂K-pim-1* or the control mice do. F1 offspring from crosses between non-transgenic mice and mice heterozygous for either the *Eμ-pim-1* or the *H₂K-pim-1* gene, were treated with a single ENU dose of 60 mg per kg body weight at day 15 after birth. The lymphoma incidence of ENU-treated and non-treated transgenic and non-transgenic mice is shown in Fig. 2. Both the *Eμ-pim-1* and *H₂K-pim-1* mice show a strongly increased incidence of lymphomas with a reduced latency period after ENU treatment when compared with non-transgenic mice. The incidence of lymphomas found in non-transgenic mice agrees with another study in which ENU and *N*-methyl-*N*-nitrosourea were used to induce lymphomas in mice³.

As MuLV-induced lymphomagenesis in *Eμ-pim-1* transgenic mice is mediated by the proviral activation of either the *c-myc* or *N-myc* gene, we determined the expression of the *c-myc* and *N-myc* genes in lymphomas induced by ENU. *N-myc* expression was not increased in any of these lymphomas (Fig. 3, panel 4). In contrast, high levels of *c-myc* mRNA were found in nearly all lymphomas. The intermediate levels of *c-myc* mRNA in lymphomas 2, 4 and 65 were still significantly elevated compared with a lymphoma having a *c-myc* mRNA level comparable to that of normal spleen (lane B). The expression of most of the lymphomas was similar to that from lymphomas in which *c-myc*

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had been activated by proviral integration (Fig. 3, panel 3: compare control lanes *D* and *E* with the other lanes). The ENU-induced lymphomas were of T-cell origin, as evidenced by the clonal rearrangements of the T-cell receptor β -chain gene (data not shown). Fluorescence-activated cell sorter analysis using T and B cell-specific cell-surface markers (not shown) showed that the ENU-induced lymphomas were phenotypically indistinguishable from T-cell lymphomas occurring spontaneously in *pim-1* transgenic mice or lymphomas induced by MuLV in non-transgenic mice. In a proportion of these latter lymphomas, we found no over-expression of *c-myc* or *N-myc* (for example, tumours *B* and *C* in Fig. 3), indicating that increased *c-myc* expression is not an intrinsic property of these cells². Therefore it is unlikely that the high expression of *c-myc* in ENU-induced lymphomas simply reflects the differentiation or growth state of the tumour cells. Rather, ENU is either a direct or an indirect cause of the increased *c-myc* mRNA levels. As carcinogenic treatment can activate endogenous retroviruses, resulting in a viraemia that, in turn, could activate proto-oncogenes by proviral insertion⁴, we determined whether replication of endogenous retrovirus had been induced. Northern blots of lymphoma RNAs were hybridized to a probe containing an intact MuLV genome. This probe, which also hybridizes with the 2.8-kilobase *pim-1* transcript in the transgenic strains because of a U3 long terminal repeat within the transgene, reveals that there are additional hybridizing viral RNAs in some of the lymphomas (Fig. 3, panel 5), but the level of expression is extremely low (compare lanes *D* and *E* of MuLV-induced lymphoma RNA with lanes 4, 22, 29, 41, 45, 55, 17 and 61 of ENU-induced lymphoma RNAs). None of the lymphomas has proviral insertions near either the *c-myc* gene or the *N-myc* gene, which was found for all MuLV-induced lymphomas in *Eμ-pim-1* transgenic mice⁵. We conclude that endogenous retrovirus activation does not play a part in ENU-induced lymphomagenesis in our *pim-1* transgenic mice. As expected, increased expression of the *pim-1* transgenes is found in lymphomas of the *Eμ-pim-1* and *H2K-pim-1* transgenic mice, with the exception of the lymphoma from the *H2K-pim-1* transgenic mouse 64, which barely expresses the transgene (Fig. 3, panel 1). Remarkably, the amount of endogenous *pim-1* expression in lymphomas from both *pim-1* transgenic and non-transgenic mice is highly variable (Fig. 3, panel 2). It is unlikely that there is selection for increased endogenous *pim-1* expression in the presence of a highly

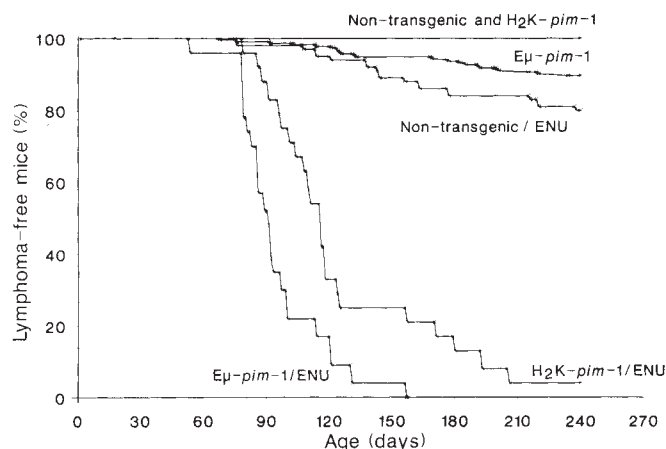


FIG. 2 ENU-induced lymphoma incidence in *Eμ-pim-1*, *H2K-pim-1* and control mice. Lymphoma-free survival of non-transgenic mice: without treatment ($n=100$) or after ENU treatment ($n=65$). Lymphoma-free survival of *Eμ-pim-1* transgenic mice: without treatment ($n=200$) or after ENU treatment ($n=23$). Lymphoma-free survival of *H2K-pim-1* transgenic mice: without treatment ($n=50$) or after ENU treatment ($n=24$).

METHODS. Lymphoma induction: Offspring of crosses between heterozygous transgenic (*Eμ-pim-1*) and *H2K-pim-1*) and C57BL/LiA mice were injected i.p. at day 15 after birth with 60 mg ENU per kg of body weight. ENU was freshly dissolved in PBS acidified with acetic acid to pH 6. Mice were examined every other day for lymphoma development, killed when moribund and the lymphomas collected for analysis.

expressed *pim-1* transgene. Probably the enhanced expression of the *pim-1* germline allele is a secondary effect of the (in)activation of other genes by ENU.

Other studies have shown that *K-ras* or *N-ras*, but not *Ha-ras*, is involved in *N*-methyl-*N*-nitrosourea-induced lymphomagenesis in mice^{6,7}, where up to 50% of the lymphomas are found to carry mutations in codon 12 in *K-ras* and in codon 12, 13, and 61 in *N-ras*. Screening mutations in codons 12, 13, or 61 of either *K-* or *N-ras* reveals that in 6 out of 12 lymphomas from non-transgenic mice, there is a mutation in *K-ras*, being in codon 12 in four cases and in codon 61 in two. By contrast, we found only three mutations, two in *K-ras* codon 12 and one in *N-ras* codon 61, in 22 lymphomas from *Eμ-pim-1* mice and

FIG. 1 Transgenic *pim-1* constructs: Germline *pim-1* genomic organization (top panel), the *Eμ-pim-1* construct² (middle) and the *H2K-pim-1* construct (bottom panel).

METHODS. DNA constructs: The construction of the *Eμ-pim-1* transgene has been described². The *H2K-pim-1* construct was derived from the *Eμ-pim-1* construct by replacing the *pim-1* promoter region entirely by the *H2K* promoter (by courtesy of Dr D. Morello, Paris). The *H2K* promoter was fused to the *pim-1* sequences at the *Pst*I site at the boundary of the most 5' *pim-1* exon. Generation of transgenic mice: The DNA fragments used for injection were released from their vectors by the appropriate restriction endonucleases and purified by agarose gel electrophoresis and electroelution. The final DNA concentration was adjusted to 4 $\mu\text{g ml}^{-1}$. Fertilized mouse eggs were recovered in cumulus from the oviducts of superovulated (CBA/BrA $\times$ C57BL/LiA) F1 females that had mated with F1 males several hours earlier. The DNA fragments were injected into the most accessible pronucleus of each fertilized egg¹⁰. After overnight culturing, two-cell-stage embryos were implanted into the oviducts of 1-day pseudopregnant F1 fosters and carried to term. Several weeks after the birth of the animals that had developed from the microinjected eggs, total genomic DNA was prepared from tail biopsies¹⁰. Transgenic founders were back-crossed with either (CBA/BrA $\times$ C57BL/LiA)F1 or the C57BL/LiA parental strain.

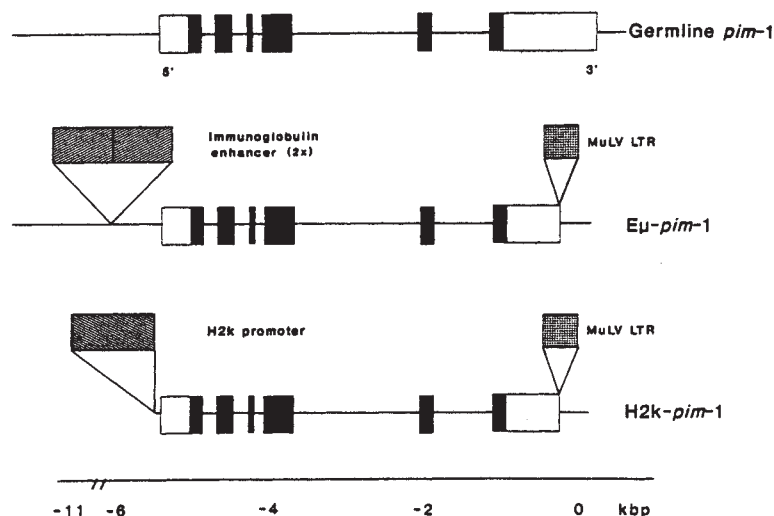
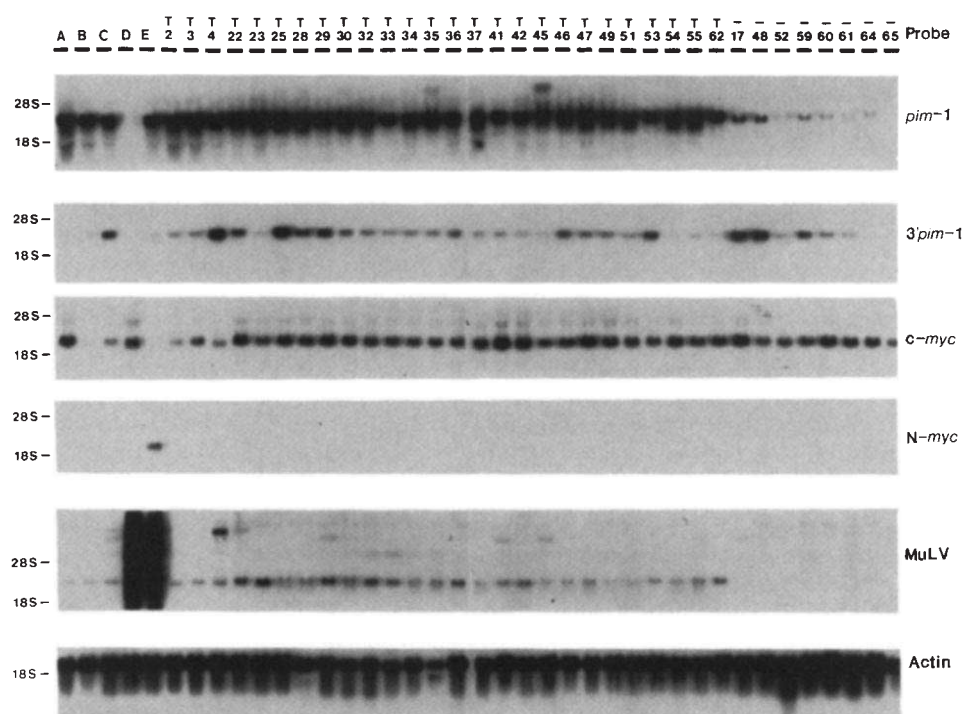


FIG. 3 Expression of *c-myc*, *N-myc*, *pim-1* and endogenous viruses, analysed by northern blotting. Lanes A-E, positive and negative controls. Lanes A, B and C, RNA isolated from spontaneous lymphomas in $E\mu$ -*pim-1* transgenic mice. Lane E, RNA isolated from a MuLV-induced lymphoma of a non-transgenic mouse. This lymphoma bears a proviral integration in the 3' untranslated region of the *N-myc* gene resulting in high expression of a slightly shorter transcript². Lane D, RNA from a MuLV-induced lymphoma of a $E\mu$ -*pim-1* transgenic mouse. In this lymphoma *c-myc* is highly over expressed because of a proviral integration in *c-myc*. Lanes denoted with a T represent tumour RNA isolated from *pim-1* transgenic mice. The numbers 2, 3, 4, 22, 23, 29, 32, 33, 36, 41, 42, 53 and 62 correspond to $E\mu$ -*pim-1* transgenic mice; 25, 28, 30, 34, 35, 37, 45, 47, 49, 51, 54 and 55, to H_2K -*pim-1* transgenic mice. Lanes headed with a dash represent lymphomas in non-transgenic littermates. One exception is 64, which corresponds to a lymphoma induced in a H_2K -*pim-1* transgenic mouse but which barely expresses the transgene. The blot was hybridized in turn to the six probes indicated to the right (ribosomal RNA size markers are shown on the left).



METHODS. For Northern blot analysis, 25 µg total RNA prepared by the LiCl-urea method was separated on 1% agarose formaldehyde gels¹¹ and transferred to nitran as recommended by the supplier. Probes used for RNA analysis: *pim-1*, probe A (ref. 1); the 3' *pim-1* probe, inserted in M13, extends from genomic map coordinate 6,619 (*Hind*III) to 6,939 (*Bgl*III)¹² and

specifically detects the endogenous *pim-1* transcript; *c-myc* and *N-myc*^{1,2}; the MuLV probe is a complete MuLV provirus¹³; actin¹⁴. Probes were ³²P-labelled by nick-translation (*pim-1*, *c-myc*, *N-myc* and MuLV) or by M13 primer extension (actin, *pim-1* endogenous-specific probe). Hybridization conditions were as described¹, with 1% SDS in all solutions and the final wash in 0.1 × SSC at 60 °C, except the MuLV hybridization, in which the last wash was at 42 °C.

only one mutation, in *N-ras* codon 61, in 18 lymphomas of H_2K -*pim-1* mice (Table 1). The lower incidence of mutations in *K-ras* or *N-ras* in lymphomas of *pim-1* transgenic mice could be explained by a reduced selective advantage conferred by a mutation in *ras* in a cell already overexpressing the *pim-1* transgene. Alternatively, in both transgenic and non-transgenic mice the percentage of *ras* mutations with respect to the number of animals treated with ENU is essentially the same (Table 1). Varying the ENU dosage should give us more information on the interaction between *c-myc*, *pim-1* and *ras* in lymphomagenesis.

Six out of ten lymphomas with a *ras* mutation lack the normal *ras* allele. Complete or partial loss of the normal allele has been reported for tumours bearing a mutated *neu*⁸, *Ha-ras*⁹, and *N-ras*⁷. The observed hemi- or homozygosity of the mutated

allele suggests that a selective advantage is associated with the loss of the normal *ras* allele during lymphomagenesis.

In conclusion, we show that *pim-1* transgenic mice represent a highly sensitive *in vivo* system for ENU-induced lymphomagenesis. The overexpression of *c-myc* in all ENU-induced lymphomas suggests that *c-myc* has a pivotal role in the generation of these tumours. The low incidence of spontaneous tumours in *pim-1* transgenic mice, coupled to a 100% lymphoma incidence after treatment with a single relatively low dose of carcinogen, indicates that *pim-1* transgenic mice could be suitable for studying the carcinogenicity of other chemicals. □

TABLE 1 Frequency of *ras* mutations in ENU-induced lymphomas in non-transgenic mice and in H_2K -*pim-1* and $E\mu$ -*pim-1* transgenic mice

	Non-transgenic	H_2K - <i>pim-1</i>	$E\mu$ - <i>pim-1</i>
Total number of mice	65	24	23
Mice without lymphoma*	52	1	0
Mice with lymphoma*	13	23	23
Mice analysed for mutation in <i>ras</i>	12	18	22
Mice with mutation in <i>ras</i>	6	1	3

To detect *ras* mutations, DNA sequences encoding the codons 12, 13 and 61 of both *K-ras* and *N-ras* were amplified by the polymerase chain reaction¹⁵ followed by oligonucleotide mismatch hybridization, performed essentially as described^{16,17}.

* Within 240 days of ENU treatment.

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Negative regulation of serum-responsive enhancer elements

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TRANSCRIPTION of the *c-fos* proto-oncogene and the cytoskeletal actin genes is induced within minutes of the addition of serum growth factors in a variety of cell types^{1,2}. Inhibitors of protein synthesis such as cycloheximide have been shown to dramatically potentiate the transcriptional response, an effect termed 'superinduction' (refs 1–3). Although the stimulatory effect of serum has been shown to be transmitted through a *cis*-acting enhancer sequence termed a serum response element (SRE)^{4,5}, the sequence element(s) responsible for mediating the effect of cycloheximide has not been identified. We now report that a synthetic copy of the *c-fos* SRE is sufficient to confer cycloheximide-dependent inducibility upon a heterologous promoter. This does not require the presence of serum, but several mutations in the SRE that impair serum-inducibility also impair cycloheximide-inducibility. These results imply that serum-responsive enhancer elements are negatively regulated by one or more labile proteins and that both positive and negative regulators of enhancer activity require a functional 'CArG box', a sequence domain previously implicated in muscle-specific transcription^{6,7}.

Although transcriptional enhancer elements exert positive effects upon linked promoters⁸, negative regulation of a variety of enhancer elements has been reported^{9–12}. To test the possibility that negative regulation of serum-responsive enhancer elements might account for the ability of cycloheximide to potentiate serum-induced transcription, a synthetic copy of the *c-fos* SRE was prepared (Fig. 1) and cloned into the *Bam*HI site upstream of the Herpes simplex virus thymidine kinase (tk) promoter in the chloramphenicol acetyltransferase (CAT) reporter plasmid pBLCAT2 (ref. 13). Similar constructs were prepared using copies of SRE-like sequences previously noted in the 5'-flanking regions of the human γ -actin¹⁴ and fibronectin¹⁵ genes and the mouse β -actin¹⁶ gene.

AKR-2B mouse fibroblasts were transfected with constructs containing a single copy of the SRE or SRE-like sequences, and the effects of serum stimulation, with or without a transient exposure to cycloheximide, were determined as described previously¹⁶. As shown in Fig. 2a, the enhancerless tk promoter was virtually inactive in either serum-starved or serum-stimulated cells as reflected by low levels of CAT activity. A small increase in CAT activity was evident when cycloheximide was included for the initial 4 h of serum stimulation, a result which may reflect a small, nonspecific stabilization effect on total messenger RNA¹⁸. In contrast, transfection with constructs containing the *c-fos* SRE (p29fos tk) or the related γ -actin sequence (p32 γ Act tk) produced high levels of CAT activity in serum-stimulated cells receiving a transient exposure to cycloheximide. Stimulation with serum alone was much less effective indicating that full elaboration of enhancer activity required the inhibition of protein synthesis.

To verify that superinduction of CAT activity reflected an increase in correctly initiated tk-CAT RNA transcripts, cells were co-transfected with p32 γ Act tk and p β Ac-CAT, a CAT construct containing the serum and cycloheximide-inducible promoter of the mouse β -actin gene¹⁶. As shown in Fig. 2b,

primer-extension analysis using a CAT coding sequence primer yielded both the 213-nucleotide-long extension product expected from the β -actin promoter¹⁶, and a 159-nucleotide-long product expected for transcripts correctly initiated from the tk promoter.

The data shown in Fig. 2 also indicate that the other SRE-like sequences tested were incapable of stimulating tk promoter activity despite substantial homology to the *c-fos* and γ -actin SREs. To delineate nucleotides essential to cycloheximide-mediated superinducibility, a series of point mutations were incorporated into the functional γ -actin sequence as shown in Fig. 3. These mutant SREs were linked to the tk promoter in pBLCAT2 and used to transfect AKR-2B cells as before. All constructs which contained a variation of the central 10-base-pair CArG motif exhibited both a reduced ability to stimulate basal levels of transcription as well as a greatly diminished ability to stimulate transcription in response to both serum and cycloheximide. Perhaps the most striking of these was γ Ac31.3 in which the deletion of a single base pair in the central AT-rich spacer completely abolished inducibility in response to both serum and serum plus cycloheximide. By contrast, mutations distal to the CArG domain, for example, γ Ac32.4, had considerably less effect.

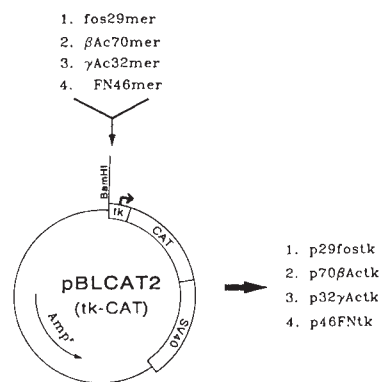
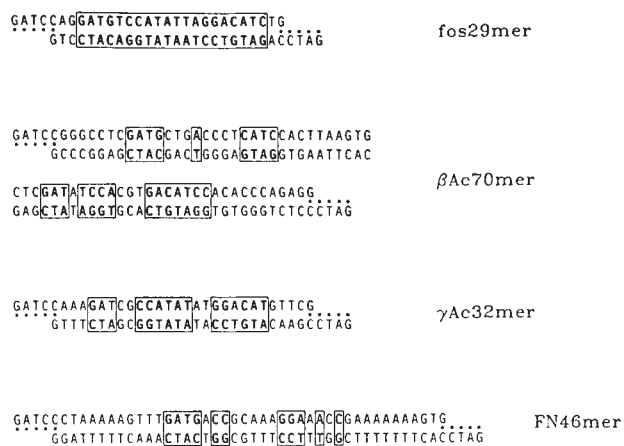


FIG. 1 Sequence of synthetic oligonucleotides and structure of tk-CAT reporter plasmids. Synthetic oligonucleotides corresponding to reported sequences were synthesized on an Applied Biosystems Model 380A DNA synthesizer and annealed to yield the indicated double-stranded sequences. Boxed regions correspond to the *c-fos* -297 to -317 dyad-symmetry element^{4,5} or to conserved sequences associated with the promoters of the mouse β -actin gene¹⁶, the human γ -actin gene¹⁴, and the human fibronectin gene¹⁵. Overlying and underlying dots denote nucleotides added to generate *Bam*HI-compatible cohesive termini. As shown in the lower portion of the figure, the double-stranded oligonucleotides were inserted upstream of the HSV thymidine kinase promoter in pBLCAT2 (ref. 13) using the *Bam*HI site of an associated polylinker region.

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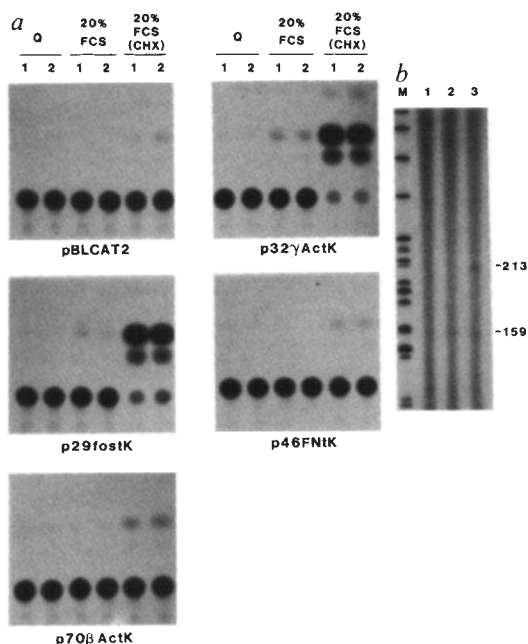


FIG. 2 Effect of serum stimulation in the absence or presence of cycloheximide on enhancer-dependent tk promoter activity in AKR-2B cells. *a* 100 mm dishes of AKR-2B mouse embryo fibroblasts were transfected in parallel with 10 μ g of the indicated plasmids using a DEAE-dextran technique¹⁷ as previously described¹⁶. Transfected cells were grown for 24 h in McCoy's 5A medium containing 5% fetal calf serum (FCS) then rendered quiescent (Q) by placing in serum-free medium MCDB 402 for an additional 36 h. Cells were either treated for 6 h with fresh McCoy's medium containing 20% FCS or for 4 h with the same medium additionally containing 10 μ g ml⁻¹ cycloheximide (CHX) followed by a 2-h washout with medium lacking cycloheximide. Cell extracts were prepared and equal amounts of total protein from each assayed for CAT activity as previously described¹⁶. Numbers above lanes denote independent transfection experiments. *b*, Cells were cotransfected with 5 μ g each of p32 γ ActK and p β Ac-CAT¹⁶ and treated as described above except that the 2 h washout with medium lacking cycloheximide was omitted. Total RNA was isolated and used for primer-extension analysis as previously described¹⁶. The primer corresponded to nucleotides 29–61 of the CAT coding sequence. Lane 1, RNA from quiescent cells, lane 2, RNA from cells treated for 6 h with 20% FCS, lane 3, RNA from cells treated for 6 h with 20% FCS plus 10 μ g ml⁻¹ cycloheximide. M, *Msp*I digest of pBR322 DNA.

The experiments shown in Figs 2 and 3 strongly imply that the serum-responsive enhancer elements typified by the *c-fos* and γ -actin sequences are negatively regulated by a labile protein(s). To distinguish between a negative feedback effect involving a serum-inducible protein¹⁹ or one which is constitutively synthesized in the absence of serum-stimulation, we studied the ability of cycloheximide to induce SRE-dependent tk promoter activity in cells maintained under completely serum-free conditions. As shown in Fig. 4*a*, treatment with cycloheximide alone induced a time-dependent increase in promoter activity consistent with the decay of a labile repressor with a half life of ~ 2.5 h. Mutations in the CARG box which eliminated inducibility by serum plus cycloheximide also eliminated inducibility in response to cycloheximide alone, whereas mutations distal to this domain again had less effect (Fig. 4*b*). Finally, serum starvation is not necessary for the stimulation of enhancer activity by cycloheximide as evidenced by a strong inductive effect in rapidly growing cells (Fig. 4*c*). These results imply that only a small fraction of the enhancers potential is realized under normal growth conditions and that both serum-starved and nonserum-starved cells synthesize a factor(s) which represses SRE-dependent transcription.

The ability of protein biosynthetic inhibitors to superinduce the transcription of growth factor-responsive genes has been recognized for several years^{1,2}, yet until now, no specific sequence element capable of transmitting this effect has been identified. Although not eliminating the possibility of additional negative control elements^{19,20}, these studies indicate that serum-responsive enhancer elements are sufficient to confer strong cycloheximide inducibility to a heterologous promoter. It is important to note, however, that mutations in the CARG domain of an SRE which abolish cycloheximide inducibility do not result in up-regulation of enhancer activity in the absence of cycloheximide. This would be the expected result if such mutations selectively impaired the binding of a labile repressor to DNA. The fact that such mutations actually impair enhancer activity under all conditions tested suggests that their primary effect may be to interfere with the binding of a positive factor required for enhancer function. Indeed, similar mutations in the CARG domain of the *c-fos* SRE have been shown to eliminate the binding of a positive-acting transcription factor termed the serum-response factor (SRF)^{21,22}. Based on these observations, we speculate that the putative repressor(s) of SRE-dependent

transcription does not interact directly with DNA but may instead exert its effects by protein-protein contact, perhaps with the SRF itself. Such a complex, though bound to DNA, would not activate transcription and would therefore be consistent with numerous studies showing that the DNA-binding activity of the

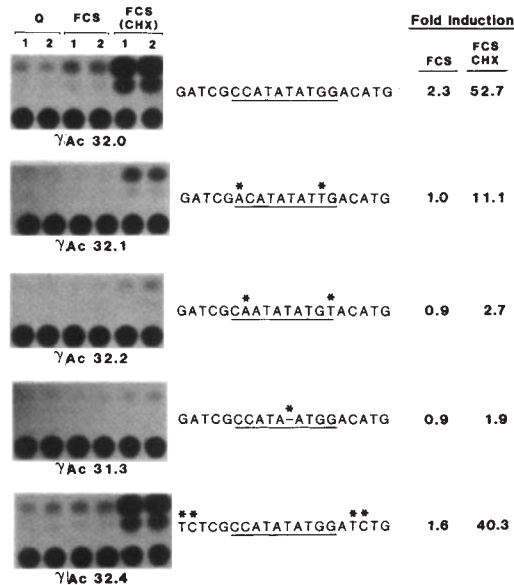


FIG. 3 Mutational analysis of the γ -Actin serum and cycloheximide-responsive element. Recombinant tk-CAT vectors analogous to those illustrated in Fig. 1 were constructed using synthetic oligonucleotides incorporating the indicated variations (*) of the γ -actin SRE-like sequence. Heterologous cohesive termini (*Sall*, *Bam*HI) were incorporated to insure unidirectional insertions bridging the polylinker *Sall* and *Bam*HI sites in pBLCAT2. AKR-2B cells, transfected in parallel with the indicated mutants, were treated exactly as described in the legend to Fig. 2. The specific activity of CAT in cell extracts was determined by liquid scintillation counting of excised sections of the illustrated thin-layer plates and expressed as a per cent conversion of ¹⁴C-chloramphenicol to its acetylated derivatives. These values were used to calculate the fold induction of CAT-specific activity in FCS or FCS plus CHX-treated cells relative to quiescent (Q) cells. The values given are the average of the two independent transfection assays denoted by the numbers above the lanes.

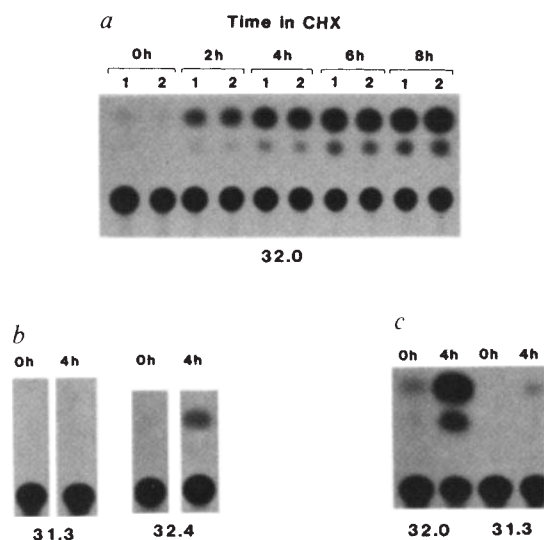


FIG. 4 Induction of γ -actin enhancer activity by cycloheximide does not require serum factors. *a*, AKR-2B cells transfected with γ Ac32.0 as described in the legend to Fig. 2 were placed in serum-free medium MCDB 402 for 36 h, then treated with $10 \mu\text{g ml}^{-1}$ CHX for the indicated times. Cell extracts were prepared following a 2-h washout with serum-free medium lacking CHX. Numbers above lanes denote independent transfection experiments. *b*, Effect of specific mutations on enhancer activity in absence of serum factors. AKR-2B cells transfected with SRE mutants γ Ac31.3 or γ Ac32.4 were treated as described in *a* above. Cycloheximide was added for the indicated times, then removed as in *a*. *c*, Induction of SRE-dependent enhancer activity by cycloheximide does not require serum starvation. Cells were transfected with either γ Ac32.0 or the indicated mutant then grown for 24 h in McCoys 5A medium containing 5% FCS. Cycloheximide was added for the indicated times followed by a 2-h washout in the same medium.

SRF is independent of serum-induced transcriptional activity^{21–26}. Although other mechanisms are plausible for example, positive and negative factors might share a common SRE binding site, it is interesting to note that at least one SRF-interacting protein has recently been identified²⁷. Although lacking the functional properties expected of a repressor, the identification of such a protein indicates that complex formation between the SRF and additional proteins may play a key role in regulating SRE-dependent transcription. □

Chimaeras of Myc oncoprotein and steroid receptors cause hormone-dependent transformation of cells

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THE human proto-oncogene *myc* encodes a nuclear phosphoprotein whose primary biochemical function is still unknown^{1,2}. To facilitate further study of that function, we have created conditional alleles of *myc* by fusing the hormone-binding domain of the human oestrogen receptor gene to the 5' or the 3' end of human *myc*. The two chimaeric genes, designated *mycer* and *ermyc*, encode proteins that bind oestrogen with high affinity. Expression of one of the genes, *mycer*, transforms a rat fibroblast cell line in a tightly oestrogen-dependent manner. Transformation is dependent on the presence of a functional *myc* gene in the chimaera and is reversible upon removal of the hormone. The chimaeric genes will be useful tools to study the mechanisms by which Myc affects cellular phenotype. Recently, chimaeras between the adenovirus E1A protein and the hormone binding domain of the rat glucocorticoid receptor were shown to activate transcription in a manner characteristic for E1A, but in a hormone regulated manner¹⁰. We therefore asked whether the same strategy could be applied to the product of *myc*.

Two chimaeric genes were constructed: one carried the oestrogen-binding domain³ at the 5' end of *myc* and was designated *ermyc*, the second, *mycer*, carried the oestrogen-binding domain at the 3' end of *myc* (Fig. 1).

When the chimaeric genes were translated *in vitro*, the translation products bound β -oestradiol with an affinity similar to that displayed by the intact oestrogen receptor ($K_m = 0.36$ nM, 3 nM and 1 nM, respectively) (Fig. 2). We estimated that between 16% (*Mycer*) and 45% (*Ermyc*) of the chimaeric proteins synthesized were competent for hormone binding. We conclude that the hormone-binding domain assumes an active conformation in these chimaeric proteins. In transiently transfected COS7 cells, both chimaeric proteins were constitutively localized in the cell nucleus (data not shown), suggesting that the nuclear localization signals of the Myc protein⁴ are still functional.

To test whether the chimaeric proteins carry out Myc-specific functions in a hormone-dependent manner, we infected Rat-1A fibroblasts with retroviruses carrying either *ermyc* or *mycer*. Overexpression of human Myc from a retroviral vector has previously been shown to transform Rat-1A cells morphologically and to induce them to grow in soft agar⁵. We found that neither high levels of β -oestradiol (up to $10 \mu\text{M}$) or of an oestrogen antagonist, hydroxy-tamoxifen (up to 100 nM), induced any obvious morphological alterations or growth in soft agar of these cells (see below). Indeed, we could not detect any oestrogen receptor in the uninfected cells, using either direct binding assays with labelled oestradiol or indirect immunofluorescence using an antibody directed against the human oestrogen receptor.

Cultures were infected with one of four different viruses: virus containing only the vector, pMV-7 (ref. 9); pMV-7-*myc*, which carries human *myc*; pMV-7-*ermyc* and pMV-7-*mycer*, which carry the respective chimaeric genes. Cells were then assayed for hormone-dependent growth in soft agar. The results of a representative experiment are summarized in Table 1.

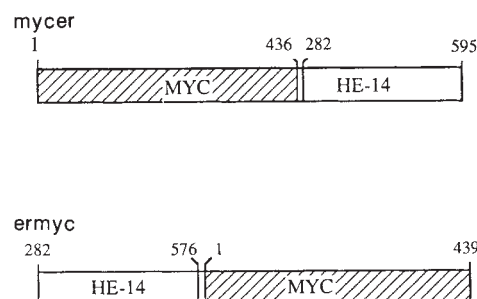
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FIG. 1 Chimaeric genes containing *myc* and regulatory domains from the oestrogen receptor gene. The human *myc* gene was used as a complementary DNA clone encompassing exons 2 and 3 of the genomic DNA⁸. hER denotes the DNA fragment encoding the hormone-binding domain of the human oestrogen receptor³. The numbers refer to the amino acids of the human oestrogen receptor or of the human *myc* protein.

METHODS. To construct the *ermyc* gene, plasmid HE-14 (ref. 3) was linearized at its unique *Bsm* site (nucleotide 2088 of the oestrogen receptor cDNA) and a *Sac*I site was introduced with the oligonucleotide 5'-GAGCTCCG-3'. An *Eco*RI-*Sac*I fragment was inserted into the polylinker of plasmid pSP65-cmyc (gift of William Lee, UCSF). This plasmid carries the human *myc* gene as an insert between the *Sma*I and the *Hind*III site of the SP65 plasmid (Promega). The cloning strategy introduces five amino acids, 5'-Glu-Leu-Ala-Pro-Thr-3' between serine 578 of the human oestrogen receptor and the initial methionine of human Myc. The chimaeric gene was inserted as an *Eco*RI-*Hind*III fragment into pMV-7 (ref. 9). The *mycer* gene was constructed by inserting a *Bam*H1 site at amino-acid position 436 into the human *myc* gene on plasmid pSP65-cmyc using the oligonucleotide 5'-GAACAGCTACGGGATCCTGTGCGTAAGG-3'; subsequently, the *Bam*H1-



*Eco*RI fragment of plasmid HE-14 was inserted into a Bluescript vector (Stratagene) so that it became flanked with a *Hind*III site at its 3' terminus. The resulting *Bam*H1-*Hind*III fragment was inserted at the 3' end of the mutated *myc* gene into plasmid pSP65-cmyc. The chimaeric gene was inserted as an *Eco*RI fragment into pMV-7.

The data demonstrate that oestradiol did not have a significant effect on the number of transformed colonies in the two control experiments containing pMV-7 and pMV-7-*myc*. In contrast, the infections with pMV-7-*ermyc* and pMV-7-*mycer* showed large increases in the number of soft agar colonies in the presence of β -oestradiol. The increase in this experiment was tenfold for the *ermyc*; for *mycer*, no colonies were observed in the absence of hormone. On average, a 12-fold stimulation in the number of colonies by β -oestradiol was found for *ermyc*. In some experiments, a small number of colonies carrying *mycer* grew in the absence of hormone. From these numbers, we estimated a 45-fold stimulation by β -oestradiol for *mycer* (average of four determinations). We conclude that the oestrogen-binding domain is capable of conferring hormone dependence on the function of the Myc protein; recently, we have also found that a similar chimaera, *mycgr*, that contains the sequence that encodes the hormone-binding domain of the rat glucocorticoid receptor fused to the 3' end of *myc* transforms these cells in a glucocorticoid-dependent manner (data not shown).

To corroborate these results, we cloned ten neomycin-resistant cell lines from colonies carrying *mycer* and growing in soft agar in the presence of hormone. All ten lines displayed a flat mor-

phology (Fig. 3) and failed to grow in soft agar in the absence of hormone. In the presence of hormone, however, the morphology was clearly transformed (Fig. 3) and the cells grew vigorously in agar (data not shown). In the presence of hormone, the cloning efficiency of these cell lines in agar was comparable to a Rat-1A cell line that had been transformed by a wild-type allele of *myc* (gift of M. Small) (data not shown).

Transformation by *mycer* is readily reversible. This was documented by two observations. First, cell lines carrying *mycer* did not grow in soft agar in the absence of β -oestradiol (data not

TABLE 1 Fusions between the oestrogen receptor and Myc confer hormone-dependent growth of Rat-1A fibroblasts in soft agar

Virus	neo ^r	Soft agar colonies		
		—	Oestrogen	OHT
pMV- <i>myc</i>	330	102	163	191
pMV- Δ <i>myc</i>	352	2	0	0
pMV- <i>ermc</i>	170	14	151	61
pMV- <i>er</i> Δ <i>myc</i>	300	0	0	5
pMV- <i>mycer</i>	230	0	138	88
pMV- Δ <i>mycer</i>	370	1	5	0

Fusions between the oestrogen receptor and Myc confer hormone-dependent growth of Rat-1A fibroblasts in soft agar. Rat fibroblasts were infected with helper-free stocks of recombinant retroviruses (generated essentially as described by Brown *et al.* ref. 9) carrying no oncogene (pMV-7), human *myc* (pMV-7-*myc*) or either of the chimaeric genes (pMV-7-*ermyc* or pMV-7-*mycer*). 24 h after infection, cells were trypsinized and divided into four aliquots: one was used to determine the number of neomycin-resistant colonies, the other three were seeded into soft agar containing 2 μ M β -oestradiol, 20 nM hydroxytamoxifen (OHT) or no hormone. Soft agar assays were carried out with medium lacking phenol-red and containing 10% charcoal-treated fetal calf serum³. Neomycin-resistant colonies were counted 7 days, colonies growing in soft agar 14 days after seeding. Δ -*myc* is a deletion mutant lacking amino-acids 106-143 (ref. 8); this mutant had previously been shown to be inactive both for transformation of Rat-1A cells and for cotransformation of primary rat embryo fibroblasts with an activated allele of *hras* (ref. 8). The result of a representative experiment is shown.

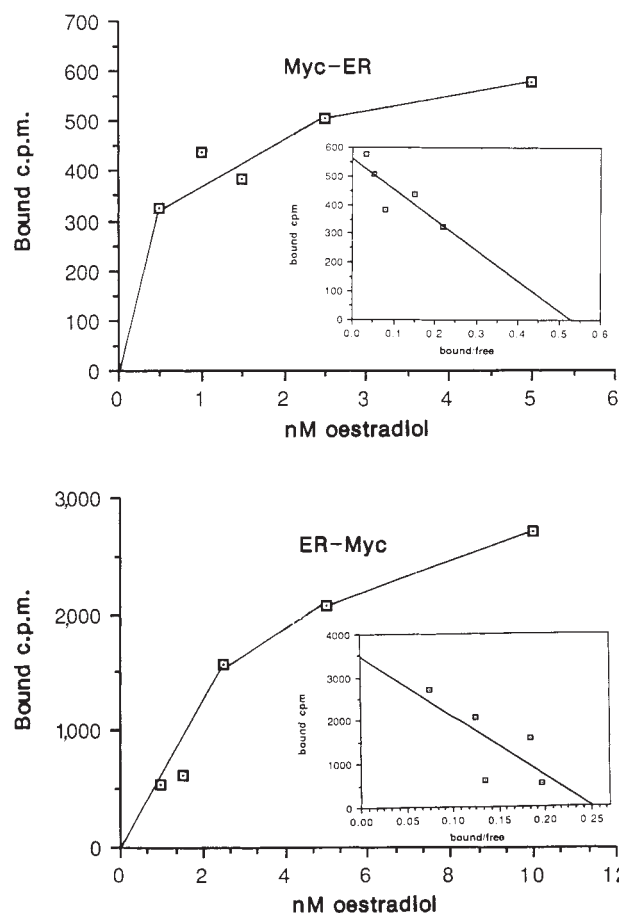


FIG. 2 Oestrogen-receptor chimaeras bind oestradiol with high affinity. The chimaeric genes were transcribed *in vitro* using SP6 polymerase and translated in a reticulocyte lysate. Aliquots of the lysate were incubated with ³H-labelled β -oestradiol overnight at 4 °C essentially as described by Kumar *et al.*³. The amount of protein synthesized was determined in a parallel reaction containing ³⁵S-labelled methionine.

shown). These lines were established from colonies grown in soft agar in the presence of β -oestradiol. Therefore, even prolonged exposure to the hormone does not cause an irreversible transformation of Rat-1A cells. Second, cells grown in the presence of hormone return to a flat morphology after reseeding into hormone-free medium (data not shown). These data support the idea that transformation by *myc* is reversible; a similar conclusion had previously been reached for macrophages using temperature-sensitive mutants of viral *myc*⁶.

The hormone-binding domain of the oestrogen receptor carries the intrinsic ability to activate transcription⁷. We were concerned that this activity might contribute to the transformation we observe with the chimaeric genes. We carried out two experiments to address this possibility. First, we used the oestrogen antagonist hydroxy-tamoxifen, which elicits binding of the oestrogen receptor to DNA but not activation of transcription⁷. The antagonist was a partial inducer of the transformation by *ermyc* and, more strikingly, *mycer* (Table 1). On average, the number of colonies in the presence of hydroxy-tamoxifen was 70% of the number in the presence of β -oestradiol ($n = 6$). Also, hydroxy-tamoxifen induced morphological transformation of cell lines carrying *mycer* (data not shown). These observations argue strongly that transcriptional activation by the oestrogen-binding domain does not contribute to transformation.

Second, we demonstrated that the transformation conferred by the chimaeric genes depends on the presence of a functional *myc* proto-oncogene in the chimaeras. To do this, we replaced *myc* in all the constructs with a deletion mutant that lacks amino-acids 106–143 of the Myc protein. This deletion mutant

has previously been shown to be functionally inactive⁸. We found that the chimaeras constructed with the mutant *myc* were indeed inactive for transformation (Table 1). We conclude that transformation by the chimaeric proteins depends on the presence of an intact Myc protein.

In summary, we have shown that chimaeric genes between the hormone-binding domains of two steroid receptors and the *myc* proto-oncogene behave as conditional alleles of *myc*. The chimaeric proteins we describe in this paper act as a new class of conditional mutants with a gratuitous regulator that does not cause side effects in the target cells. They should allow both a careful timing and titration of Myc activity and, thus, provide a useful tool for studying the mechanisms by which Myc affects cellular phenotype. □

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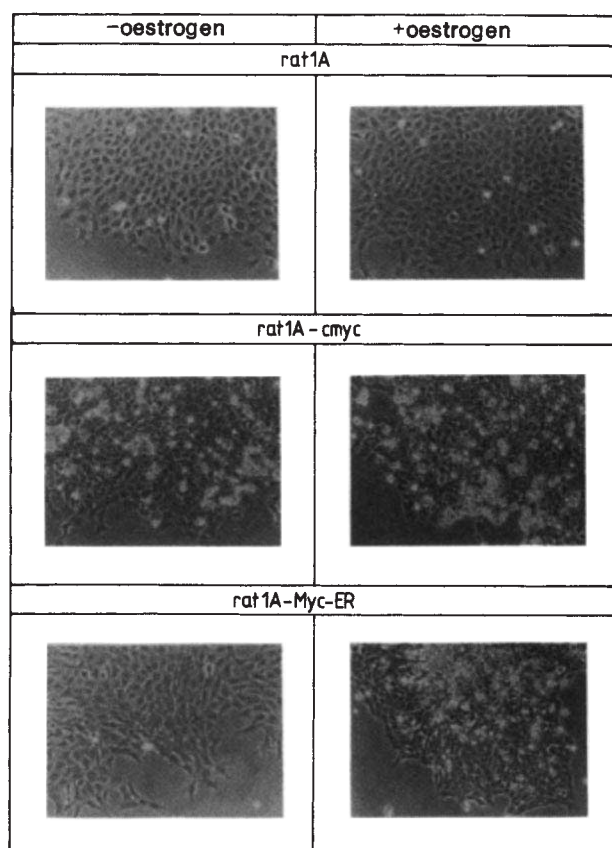


FIG. 3 Oestrogen-dependent morphological transformation of Rat-1A fibroblasts. Uninfected Rat-1A fibroblasts, a line carrying a human *myc* gene under the transcriptional control of a retroviral long terminal repeat (LTR) and one of the lines infected with the pMV-7-*mycer* virus were seeded into phenol red-free DMEM21 medium containing 10% charcoal-treated³ fetal calf serum in the presence or absence of $1 \mu\text{M}$ β -oestradiol. The photographs were taken 3.5 days after seeding.

Occupation of the *c-fos* serum response element *in vivo* by a multi-protein complex is unaltered by growth factor induction

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RAPID, transient induction of the human *c-fos* proto-oncogene by extracellular signals requires the presence in *cis* of the serum response element (SRE)^{1,2}. Two protein factors that bind to the SRE *in vitro* are the serum response factor (p67^{SRE})^{3–5} and polypeptide p62 (ref. 6). These polypeptides must interact with one another and the SRE for efficient serum induction of the *c-fos* gene⁶. Here we use dimethyl sulphate genomic footprinting⁷ to establish the *in vivo* protein contacts on the SRE and flanking sequences. In human A431 cells the patterns of protection and hyper-reactivity that we find are consistent with the presence of p67^{SRE}, p62, and at least one other protein immediately 3' to p67^{SRE}. The protein-DNA contacts we observe within the SRE are present before induction by epidermal growth factor and are unchanged during gene activation and subsequent repression. Our results indicate that a specific DNA-protein architecture may be maintained at the *c-fos* SRE, regardless of changes in the transcriptional state of the gene. Such established structures could be important generally in rapid transcriptional responses to extracellular signals.

Figure 1a and b shows the *in vivo* guanine N-7 reactivity patterns of genomic DNA produced by treatment of A431 cells with dimethyl sulphate (DMS) before, during and after *c-fos* induction by epidermal growth factor (EGF). By comparing samples reacted *in vivo* with protein-free controls, regions showing protection from methylation and enhanced methylation can be distinguished. Reactivity patterns in the SRE are therefore

FIG. 1 DMS reactivities of the *c-fos* promoter in A431 cells. *a*, Upper strand guanine ladders of genomic DNA isolated from DMS-treated A431 cells. DNA was isolated at 0, 20, 60, 120 and 240 min after addition of EGF to serum-starved cells (lanes 2, 3, 4, 5 and 6 respectively). Lane 1 contains protein-free genomic A431 DNA (G) and lane 7 *c-fos* containing plasmid DNA (P), both treated with DMS *in vitro*. The sequence of the DSE and flanking regions is shown; arrows indicate the dyad symmetry. Large arrowheads represent strong protection from DMS methylation by protein, and the smaller arrowheads denote weaker protection. Enhanced guanine N-7 reactivity is denoted by circles. The asterisk marks an enhancement and the open triangle a protection, which appear after EGF induction of the gene. The thymidine residue at position -300 is indicated for reference. *b*, As *a*, except that the lower strand is analysed. Strong methylation protection, similar to that of the G-string beginning at position -328, is found in the chicken β -globin²² and the rat *TAT* genes²³, but no function has been reported for this sequence in *c-fos* transcription. *c*, Northern blot analysis of total RNA isolated from A431 cells after EGF treatment. Lane 1 shows RNA from normally growing (NG) A431 cells. RNA was isolated at 0, 20, 60, 120 and 240 min after addition of EGF to serum-starved A431 cells (lanes 2, 3, 4, 5 and 6 respectively). *d*, Summary of *in vivo* protection from and enhancement of DMS reactivity in protein-bound, as compared with protein-free, samples. Symbols as in *a*.

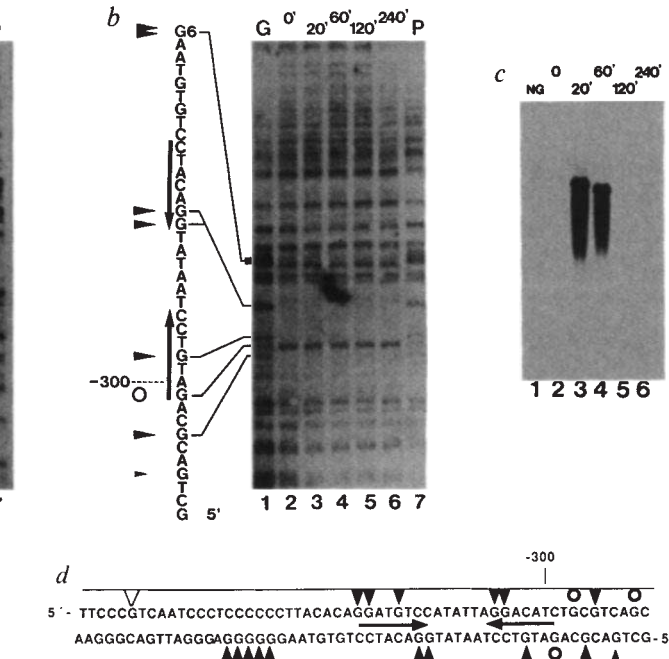
METHODS. A431 cells grown to near confluency in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) were starved for 24 h in DMEM/0.5% FCS and an additional 4 h in DMEM/0% FCS. The *c-fos* gene was then induced by the addition of human recombinant EGF (a gift from Chiron Corp.) to a final concentration of 200 ng ml⁻¹. Two minutes before the times indicated, medium was removed from the cells and mixed with HEPES buffer pH 7.4 to 10 mM and DMS to 0.1%. This mixture was then returned to the cells, which were further incubated for

unchanged by the transcriptional state of the gene, because northern analysis showed strong induction of the gene 20 min after EGF addition, with a slight decrease by 60 min and repression at 2 h (Fig. 1c).

In the dyad symmetry element (DSE) of the SRE centred at -308, two sets of three symmetrically equivalent guanine residues on the upper and lower strands are completely protected from reactivity with DMS. In addition, two guanines at positions -318 and -319 on the upper strand are not reactive. (These data are summarized in Fig. 1d.) The guanine pairs at the centre of the DSE interfere with the *in vitro* binding of p67^{SRE} when methylated³⁻⁵, and methylation of the guanine residues on the upper 5' end prevents binding of p62 (ref. 6). This protein factor does not bind alone, but requires DSE-bound p67^{SRE} for specific contacts with these residues.

The sequence 5'-TGCCTCA-3' immediately downstream of the DSE resembles the consensus binding sites for the transcription factors Ap1 and ATF/CREB (ref. 8). This region around -295 has also been implicated in negative regulation of the *c-fos* gene through Fos protein autoregulation⁹⁻¹¹. Two hyper-reactive and three protected regions are seen here *in vivo* (Fig. 1a and b). No protein has so far been found that interacts *in vitro* with the sequence around -295 in the presence of DSE-bound p67^{SRE}, but an identical sequence implicated in liver-cell specific expression of the rat tyrosine aminotransferase (TAT) gene gives, the same DMS reactivity pattern in FTO cells (G. Schütz, personal communication). It is therefore possible that these contacts in A431 and FTO cells could be produced by unidentified homologous proteins.

Several alterations in DMS reactivity are seen elsewhere in the promoter after gene induction (Fig. 1a). One hour after



2 min at room temperature. After two PBS washes, the cells were lysed with SDS and their DNA isolated. *In vitro* methylation of protein-free DNA has been described²⁴. 25 μ g samples were restricted with *Bss*HI (Biolabs), piperidine-treated and separated on 8% sequencing gels. The next steps of genomic footprinting were performed as described⁷, with the following modification: single-stranded DNA probes were synthesized according to ref. 25 with RNA generated from Bluescribe vectors (Stratagene) containing sequences from -96 to -222 of the human *c-fos* gene. Filters were exposed for 14-15 days with intensifying screens. Total RNA was isolated from A431 cells at the indicated times after EGF induction. Northern blots²⁶ were analysed with RNA probes generated from plasmids containing the third exon of the human *c-fos* gene. Loadings of RNA were the same in each lane, as confirmed by visualization of RNA on the nitrocellulose filter by ultraviolet light shadowing.

induction, there is enhanced reactivity in the upper strand guanine residues -351/-353. A guanine residue at -342 on the upper strand becomes more protected 20 min after EGF addition to the cells. Treatment of mouse cells with *v-sis* conditioned medium results in induced binding of a protein factor to this sequence *in vitro*¹².

The changes in reactivity observed in the *v-sis* conditioned medium responsive element on induction, as well as the RNA analysis, confirm the ability of A431 cells to respond to EGF. Furthermore, the individual *c-fos* genes in the system are probably in a uniform state, as indicated by the complete lack of DMS reactivity in the DSE. This inference is corroborated by indirect immunofluorescence with anti-Fos antibodies, which shows that EGF treatment induces >95% of A431 cells to produce Fos protein (ref. 13, and data not shown).

In gel retardation assays with HeLa cell extracts and SRE-containing oligonucleotides, two specific shifts are caused by p67^{SRE} alone and p62 in combination with p67^{SRE} (ref. 6). These same complexes are formed with extracts from starved or EGF-treated A431 cells (data not shown). To extend our correlation of *in vivo* DMS reactivity with these proteins on the SRE, we studied methylation protection by A431 extracts *in vitro*. DNA complexed by p67^{SRE} alone (Fig. 2, lanes 2 and 7) is protected from methylation on only the two sets of three symmetrically equivalent upper and lower strand guanine residues of the DSE. In addition, there is an enhancement of the lower strand guanine at position -299. The complex including p62 (Fig. 2, lanes 1 and 8) gives additional protection of the upper-strand guanines at positions -318 and -319; the corresponding patterns are also present *in vivo*. The *in vitro* and *in vivo* results are summarized in Fig. 3. The pattern of DMS reactivity within the SRE in A431

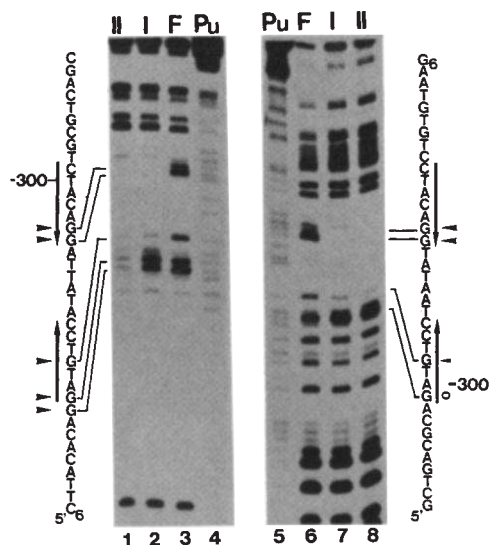


FIG. 2 *In vitro* methylation protection of SRE-containing fragments by A431 nuclear extracts. Upper (lanes 1–4) and lower (lanes 5–8) strand reactivities are shown. Complex II contains p67^{SRF} and p62 (lanes 1 and 8); complex I has only p67^{SRF} (lanes 2 and 7). DNA free of bound protein (F) is shown in lanes 3 and 6. Purine ladders (Pu) are indicated for reference (lanes 4 and 5). The sequence of the DSE and surrounding region is indicated. Symbols as in Fig. 1.

METHODS. DNA fragments spanning the SRE were labelled with polynucleotide kinase and incubated in A431 nuclear extracts, prepared as described²⁷, in the presence of 0.9 mg ml⁻¹ poly d(I-C) and 0.75 mg ml⁻¹ salmon sperm DNA. After 10 min on ice, the complexes were treated with DMS (1.25%) for 2 min at room temperature. Reactions were stopped by addition of dithiothreitol to 25mM and loaded on to a 5% polyacrylamide gel to separate complexes I and II. Subsequent steps have been described for methylation interference⁵.

cells leads us to conclude that it is continuously occupied by at least three protein factors: p67^{SRF}, p62 and another as-yet-unidentified factor 3' of the DSE. Multi-protein complex formation is known to be important in the regulation of other eukaryotic genes^{14–16}.

In contrast to other systems^{17–20}, the DMS footprint of all three factors remains unchanged, regardless of the transcriptional activity of the gene, so it is unlikely that the determining step in EGF induction of the *c-fos* gene in A431 cells is the induced binding of a factor to the SRE, as has been previously reported²¹. It is possible that polypeptide exchange could be occurring so rapidly as to escape detection here, but the protein-DNA contacts would have to be identical before and after

exchange. Rather, we prefer a situation in which the protein-DNA configuration over the SRE is established before induction. As subsequent repression also does not alter these direct protein-DNA contacts, the rate-limiting processes in *c-fos* activation and inactivation could involve modification to protein components of the established structure, interaction of other factors with the complex, or both. The continuous presence of a specific DNA-protein architecture over pivotal promoter elements may be generally used by rapid-response genes. □

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Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting

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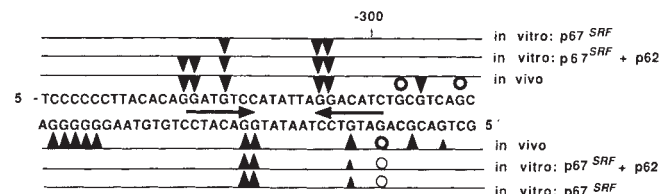


FIG. 3 Summary of *in vivo* and *in vitro* DMS methylation protections and enhancements on protein-bound, as compared with protein-free samples. Sequences from positions -290 to -333 of the *c-fos* gene are shown. *In vivo*, indicates *in vivo* protection against and enhancement of DMS reactivity; *in vitro*: p67^{SRF} + p62, indicates *in vitro* DMS protection in complex II, which contains p67^{SRF} and p62 (ref. 6); *in vitro*: p67^{SRF}, indicates protection in complex I, which contains p67^{SRF} (ref. 6). Symbols as in Fig. 1.

CELLULAR resistance to insulin caused by a reduction in insulin-mediated glucose uptake can be produced in rats by chemically inducing diabetes with streptozotocin and by fasting^{1–3}. Two glucose transporter isoforms are expressed in fat cells: (1) the insulin-responsive species^{4–8} which is found only in fat and muscle, and (2) a species corresponding to the erythrocyte/Hep G2/rat brain transporter^{9,10}. We show here that fat cells isolated from streptozotocin diabetic rats and from fasted rats show a significant (60–80%) decrease in the amount of immunologically detectable insulin-sensitive glucose transporter and no change in the level of the Hep G2/rat brain transporter. Administration of insulin and refeeding, respectively, result in a return of the insulin-sensitive glucose transporter to levels that are normal or slightly above normal. Thus, peripheral tissue insulin resistance could be due to the specific reduction in the amount of insulin-sensitive glucose transporter.

Previous measurements of total glucose transporters in adipocytes from streptozotocin-induced diabetic and fasted rats have shown that the microsomal pool of intracellular glucose transporters is reduced concomitantly with the development of insulin resistance¹⁻³. Insulin-dependent movement of the intracellular transporter pool to the cell surface accounts for a major part of the effect of the hormone on cellular glucose uptake^{4,11-13}. Recent evidence supports the notion that this pool is comprised of two distinct transporter species^{4-10,14-16}. We therefore examined the effects of streptozotocin-induced diabetes on the expression of these glucose transporter isoforms in rat adipocytes. Microsomal membranes were prepared from the fat cells of normal and diabetic animals as described in the legend to Fig. 1 and were analysed for transporter by immunoblotting with either monoclonal antibody 1F8, specific for the insulin-sensitive glucose carrier⁴, or an antiserum to the human erythrocyte glucose transporter recognizing the Hep G2/rat brain species^{9,10}. The data shown in Fig. 1a demonstrate a significant (80%) decrease in the level of insulin-sensitive transporter protein in the adipocytes of the diabetic animals (streptozotocin+) when compared with that of the controls (streptozotocin-), with essentially no change in the erythrocyte/Hep G2 transporter expression under these conditions (Fig. 1b).

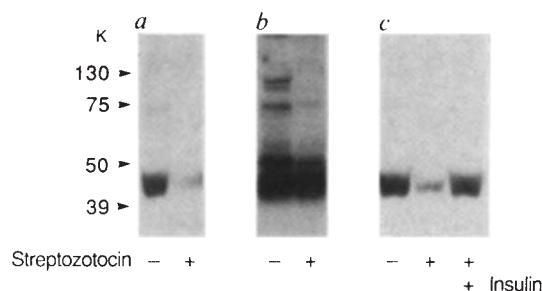


FIG. 1 Effect of diabetes and insulin on expression of glucose transporters in rat adipocytes. *a*, Immunoblot of total rat adipocyte microsomes (100 µg protein per lane) from normal and diabetic animals (streptozotocin+) using affinity-purified monoclonal antibody 1F8. *b*, As in *a*, except that antiserum to the human erythrocyte glucose transporter was used for immunoblotting. *c*, immunoblot analysis of total adipocyte microsomes (100 µg protein per lane) using monoclonal antibody 1F8 for normal rats, diabetic rats and diabetic rats receiving insulin therapy (+ Insulin). The c.p.m. in each condition (less background) in each lane from left to right are: *a*, 4,988 and 1,058; *b*, 1,396 and 1,258; *c*, 4,866, 1,192 and 4,666.

METHODS. Sprague-Dawley rats (CD strain, Charles River Breeding Laboratories, Wilmington, Mass.) were made diabetic by being given a single injection of freshly prepared streptozotocin (65 mg per kg) and were fed freely for seven days. On day seven, the animals were killed, epididymal fat pads were removed and adipocytes isolated by the method of Rodbell¹⁹. The adipocytes were homogenized for 10 s in ice-cold buffer containing 20 mM HEPES, 1 mM EDTA, 0.25 M sucrose, pH 7.4. Homogenates were centrifuged at 15,000g for 20 min at 4 °C. Total microsomes were obtained by centrifuging the supernatants at 200,000g for 90 min at 4 °C. Microsomes were resuspended in homogenization buffer and protein in the samples was determined by the method of Bradford²⁰. SDS-PAGE was performed on microsomal protein as described by Laemmli²¹. Electrobloeting of proteins to nitrocellulose, blocking and autoradiographic detection of transporter signal was performed as previously described⁴, using iodo-goat anti-mouse IgG (Dupont, NEN) for 1F8 and iodo-affinity-purified protein A (Amersham) for the anti-erythrocyte glucose transporter antibody (a gift of Dr C. Carter-Su, University of Michigan). The immunoblotting signal is linear for both antibodies for between 5–200 µg membrane protein, and the values given are after cutting, counting and subtraction of background (155–322 c.p.m.) from an area of each gel lane with no apparent radioactive band. For the data in *c*, animals were made diabetic by a single injection of streptozotocin. On day seven, one group of diabetic animals began receiving subcutaneous infusion of insulin (3 units per day) through an insulin pump, whereas another group received no further treatment. The control animals received neither streptozotocin nor insulin. On day four of the insulin therapy, the rats were killed. Adipocyte microsomal preparations and immunoblot analysis were performed as described above. This and the following experiments were performed independently 3–5 times with equivalent results.

Another group of animals was made diabetic by streptozotocin injection, and on day seven, half of the animals received insulin subcutaneously from an implanted pump. Streptozotocin treatment increased plasma glucose levels from 63 mg dl⁻¹ to 267 mg dl⁻¹, and insulin therapy lowered blood glucose levels to 93.4 mg dl⁻¹ after four days, at which time microsomes were prepared and immunoblot analysis was performed. As shown in Fig. 1c, insulin treatment of the diabetic rats caused a re-expression of the insulin-sensitive glucose transporter to levels similar to normal control. No change in expression of the erythrocyte glucose transporter was seen under these conditions (data not shown).

We then examined the effects of fasting on the expression of the insulin-sensitive and erythrocyte/Hep G2 glucose transporters, and compared this with animals fed freely throughout the experiment. Adipocytes were isolated from control and fasted rats and immunoblot analysis was performed as in Fig. 1. The data in Fig. 2a and 2c show a time-dependent decrease in expression of the insulin-sensitive transporter during the course of the fast, declining to 40% of the control level by day three. By contrast, Fig. 2b and 2c demonstrate that expression of the erythrocyte transporter in adipocytes remains essentially constant during the fast. Animals were then fasted for three days and re-fed for varying lengths of time, whereas control animals were fed freely throughout the experiment. At the times indicated, immunoblot analysis was performed on isolated microsomes. Figure 3 shows that the expression of the insulin-responsive transporter declined by >50% after a 3-day fast (0 days re-fed), and re-expression of the transporter occurred after one day of refeeding and continued to increase, upon refeeding, for seven days. At this time, the transporter was expressed at a

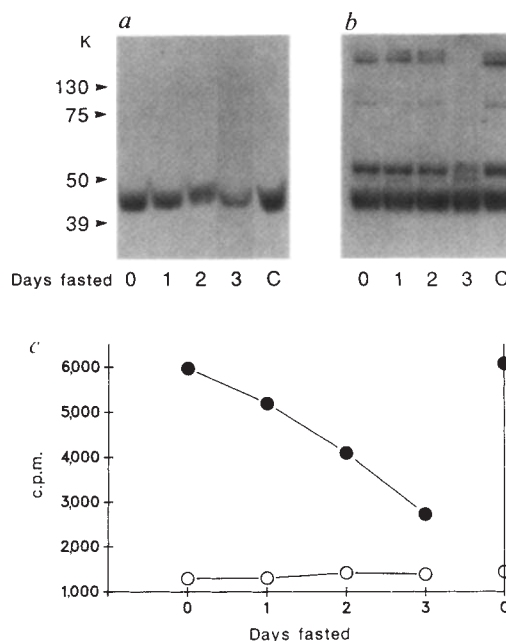


FIG. 2 Effect of fasting on the expression of glucose transporters in rat adipocytes. *a*, Immunoblot analysis of total rat adipocyte microsomes (100 µg protein per lane) using monoclonal antibody 1F8: 0, rat adipocytes, animals killed before fast; 1, day-1 fasted rat adipocytes; 2, day-2 fasted rat adipocytes; 3, day-3 fasted rat adipocytes; C, fed rat adipocytes, animals killed on day 3. *b*, Immunoblot analysis of total rat adipocyte microsomes (100 µg protein per lane) using antisera to the erythrocyte glucose transporter. Order of the samples is the same as in *a*. *c* Quantitation of transporter bands corresponding to the Hep G2 (○) and insulin-sensitive (●) transporter isoform by cutting and counting as described in the legend to Fig. 1.

METHODS. Animals were fed freely for several days. Food was then removed for a period of up to three days. Control animals were fed freely throughout the experiment. Adipocyte microsomes were prepared and immunoblot analysis was performed as described in Fig. 1.

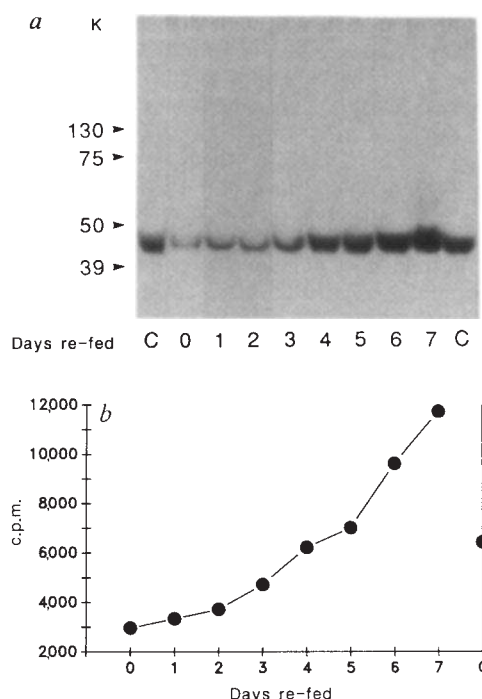


FIG. 3 Effect of refeeding on expression of the insulin-sensitive glucose transporter in adipocytes of fasted rats. *a*, Immunoblot analysis of total adipocyte microsomes (100 µg protein per lane) using affinity-purified monoclonal antibody 1F8. Rats were fasted for three days and then re-fed for the number of days indicated. The control rat adipocytes (C) in the left-hand lane were from animals killed before the fast; the controls in the right-hand lane were from animals fed throughout the experiment. *b*, quantitation of the transporter band by cutting and counting.

METHODS. Animals were fed freely for several days. Food was then removed for a period of three days. Animals were then re-fed freely for the remainder of the experiment. Control animals were fed freely throughout the entire experiment. Adipocyte microsomal preparations and immunoblot analysis were performed as described in the legend to Fig. 1.

level 1.9-fold higher than the control level, as normalized for total protein³ or 2.9-fold higher, as normalized for cell number³.

The data presented above and in the following paper¹⁷ demonstrate a specific reduced expression of the insulin-sensitive glucose transporter and its corresponding messenger RNA in adipocytes of streptozotocin diabetic rats. Expression of the Hep G2/rat brain transporter isoform remains constant under these conditions. Insulin therapy of diabetic animals leads to a renewed expression of the insulin-sensitive transporter at levels similar to those found in adipocytes of control animals. Likewise, fasted animals showed reduced expression of only insulin-sensitive transporter mRNA and protein, and re-feeding resulted in re-expression of this transporter isoform. We have recently used cytochalasin B photoaffinity-labelling of fat cell microsomes followed by immunoprecipitation with antibody 1F8 to show that the insulin-sensitive transporter comprises ~90% of the microsomal pool¹⁶. These data and that shown here (and in ref. 17) support the conclusion that the insulin-sensitive glucose transporter is the primary form of carrier responsible for insulin-induced glucose uptake in insulin-responsive tissue. In the two rodent models of insulin resistance where insulin-responsive glucose transport is compromised, only the insulin sensitive transporter isoform diminishes. In humans, the diminished expression of this protein is also likely to be responsible for the 'insulin resistance' observed in type II diabetes, where a specific decrease in the intracellular transporter pool comparable to that in the streptozotocin rat has been reported.¹⁸ Finally, as noted by Sivitz *et al.*¹⁷, it is also likely that insulin is the major regulatory factor effecting transporter expression in both

diabetes and fasting, as these conditions have opposite effects on blood glucose levels. □

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Regulation of glucose transporter messenger RNA in insulin-deficient states

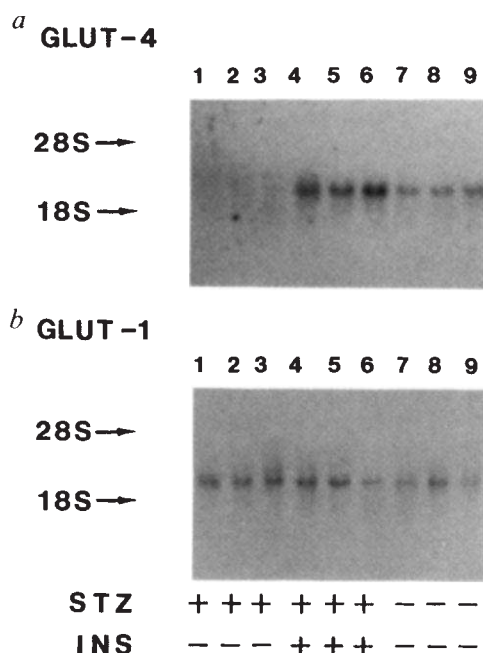
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RECENT studies have indicated that a family of structurally related proteins with distinct but overlapping tissue distributions are responsible for facilitative glucose transport in mammalian tissues^{1–13}. Insulin primarily stimulates glucose transport by inducing the redistribution of a unique glucose transporter protein from an intracellular pool to the plasma membrane³. This 509-amino-acid integral membrane protein, termed GLUT-4 (ref. 2), is the main insulin-responsive glucose transporter in adipose and muscle tissues^{1–3}. We have observed a dramatic decrease (tenfold) in the steady-state levels of GLUT-4 messenger RNA in adipose tissue from fasted rats or rats made insulin deficient with streptozotocin. Insulin treatment of the streptozotocin-diabetic rats or refeeding the fasted animals causes a rapid recovery of the GLUT-4 mRNA to levels significantly above those observed in untreated control animals. By contrast, the levels of the erythrocyte/HepG2/rat brain-type glucose transporter mRNA remain essentially unchanged under these conditions. These data suggest that the *in vivo* expression of GLUT-4 mRNA in rat adipose tissue is regulated by insulin.

Adipocytes isolated from fasted rats or from rats made insulin-deficient by destruction of the insulin-producing β -cells with the drug streptozotocin (STZ) have normal basal glucose transport activity but impaired insulin-stimulated transport^{14–16}. Moreover, as described by Berger *et al.*¹⁷, the relative amount of the insulin-responsive glucose transporter protein available for translocation to the plasma membrane is substantially decreased. We therefore examined the expression of GLUT-4 mRNA in adipose tissue from fasted rats or rats made insulin-



deficient with STZ. Four days after STZ treatment, the amount of the GLUT-4 mRNA in adipose tissue was barely detectable by northern blot analysis (Fig. 1a, lanes 1-3) and was tenfold lower than the levels present in RNA preparations from untreated control animals (Table 1; Fig. 1a, lanes 7-9). Insulin treatment of the STZ-diabetic rats for 18 h resulted in an 18-fold increase in GLUT-4 mRNA levels compared with non-insulin treated STZ-diabetic animals (Table 1; Fig. 1a, lanes 4-6). In fact, the recovery of GLUT-4 mRNA levels in response to insulin resulted in a two-fold overcompensation (Table 1). The insulin induction occurred with a half-maximal effect at 7 h and maximal stimulation within 12-24 h (Fig. 2). By contrast, STZ-induced insulin deficiency had little or no effect on the relative

FIG. 1 Expression of the GLUT-4 (a) and GLUT-1 (b) glucose transporter mRNAs in adipose tissue from control, STZ-diabetic and insulin-treated STZ-diabetic rats. Three male Sprague-Dawley rats (175-200 g) were given a single intraperitoneal injection of normal saline (lanes 7-9) and six were given a single intraperitoneal injection of STZ (Upjohn) to induce a diabetic state (lanes 1-6). Rats were considered diabetic only if the tail blood glucose values exceeded 300 mg per 100 ml. After 3 days, the STZ-diabetic rats (average weight loss of 18 ± 2 g) remained untreated or received 3 units of human regular insulin (INS) plus 4 units of intermediate acting human NPH-insulin (Lilly) by subcutaneous injection 18 h before killing.

METHODS. Epididymal fat pads were excised, washed quickly, homogenized in guanidine isothiocyanate buffer and centrifuged through cesium chloride²⁴. Total cellular RNA (30 μ g per lane) was size-fractionated on a 1% agarose/formaldehyde gel and transferred to nitrocellulose paper. The paper was hybridized to the nick-translated pHJT-3 which encodes the human GLUT-4 protein² or the prGT-1 which encodes the rat GLUT-1 protein⁹ at 42 °C in a solution of 10% dextran sulphate, 40% deionized formamide, $4 \times$ SSC, $5 \times$ Denhardt's, 0.02 mg ml^{-1} sonicated/denatured salmon testes DNA and 0.2 M Tris, pH 7.4 containing 1.5×10^7 c.p.m. ml^{-1} probe. The blots were washed three times for 5 min each in $2 \times$ SSC plus 0.1% NaDodSO₄ at room temperature and three times for 30 min each in $0.1 \times$ SSC plus 0.1% NaDodSO₄ at 55 °C followed by autoradiography at -70 °C. Because the GLUT-1 and GLUT-4 cDNA probes have <68% nucleotide sequence identity and based on the unique tissue distributions of the transcripts identified with these probes^{2,9}, they do not cross-hybridize under these moderately stringent conditions.

levels of the erythrocyte/HepG2/rat brain-type glucose transporter (GLUT-1) mRNA which is also expressed in rat adipose tissue (Fig. 1b). In addition, insulin treatment of the STZ-diabetic animals had no consistent effect on the GLUT-1 mRNA levels (Table 1; Figs 1, 2).

Eight hours of food restriction also decreased the amount of adipose tissue GLUT-4 mRNA (Fig. 3a, c). The GLUT-4 mRNA levels were maximally decreased (ninefold) by 24 h and did not further decline over the next 48 h. Refeeding (Fig. 3a, c) resulted in a more rapid recovery of the GLUT-4 mRNA ($t_{1/2} = 3$ h) than that observed for insulin treatment of the STZ-diabetic rats ($t_{1/2} = 7$ h). This presumably reflects a slower rate of exogenous insulin entry into the circulation compared to endogenous

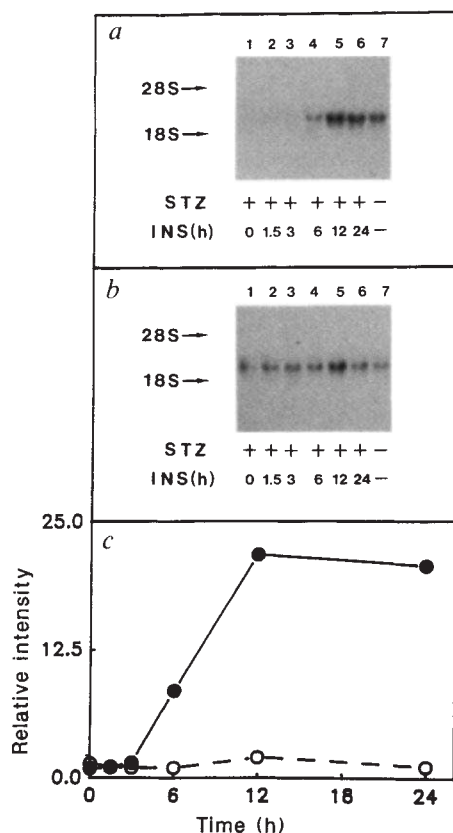
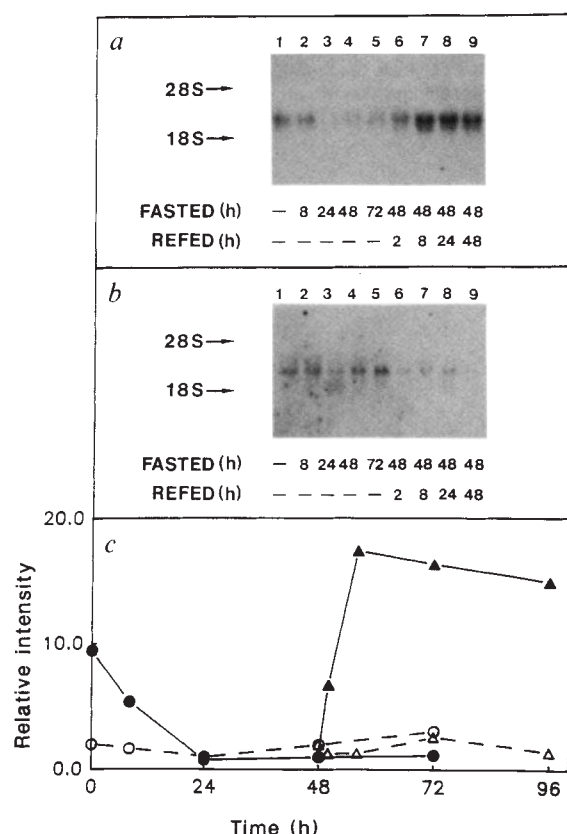


FIG. 2 Effect of insulin on the expression of the adipose tissue GLUT-4 (a) and GLUT-1 (b) glucose transporter mRNAs in STZ-diabetic rats. c, The relative abundance of the GLUT-4 (●) and GLUT-1 (○) transcripts determined by laser scanning densitometry. Male Sprague-Dawley rats were made acutely diabetic for 3 days as described in Fig. 1. The STZ-diabetic rats received a subcutaneous injection of 4 units human NPH-insulin plus 3 units of human regular insulin at 8:00 a.m. and were killed at time 0 (lane 1), 1.5 (lane 2), 3 (lane 3), 6 (lane 4), 12 (lane 5) and 24 h (lane 6). The 24-h insulin-treated animals received a second dose of 3 units NPH-insulin plus 2 units regular insulin at 12 h. At these doses of insulin, the circulating glucose levels decreased from an average baseline value of 404 ± 13 mg per 100 ml ($n=18$) to 140 ± 33 mg per 100 ml ($n=12$) during the first 3 h and averaged 197 ± 33 mg per 100 ml ($n=18$) during the rest of the experimental period. Saline-injected control animals were also killed after 24 h (lane 7). Northern blot analysis of total epididymal adipose tissue RNA was performed as described in Fig. 1 using the nick-translated GLUT-4 or GLUT-1 cDNA probes. This is a representative experiment which was independently performed three times.



insulin which is released into the portal circulation in response to refeeding. The relative amount of the GLUT-4 mRNA was maximally increased 8 h after refeeding and was about two-fold greater than the levels in adipose tissue from untreated control rats. By contrast, GLUT-1 mRNA levels were relatively unaffected by fasting/refeeding (Fig. 3b, c). Thus, the circulating glycaemic state of the animals is not a major factor in the regulation of the adipose tissue GLUT-4 glucose transporter mRNA, as blood glucose levels are reduced during periods of fasting but increased in diabetes. But these data are consistent with a direct effect of insulin which is reduced in both states of STZ-diabetes and fasting.

Recently, Kahn *et al.*¹⁸ reported that the levels of the GLUT-1 mRNA in isolated rat adipocytes remain relatively unchanged when normalized for the amount of total cellular RNA. But as fasting resulted in a 65% decrease in total cellular RNA, there was a threefold decrease in GLUT-1 mRNA abundance per cell¹⁸. Thus, the fasting-induced decrease in the amount of GLUT-4 mRNA would be markedly greater than that indicated in Fig. 3 if expressed per cell—that is, about 30-fold.

Thus, STZ-induced diabetes and fasting markedly reduce the expression of the major insulin-responsive glucose transporter

TABLE 1 Effect of STZ-induced diabetes and insulin (INS) treatment on the glycaemic state and levels of GLUT-4 and GLUT-1 mRNA in epididymal adipose tissue of the rat

Treatment	GLUT-4		GLUT-1	
	mRNA*	Glucose (mg 100 ml ⁻¹)	mRNA*	Glucose (mg 100 ml ⁻¹)
Untreated	1.0 (n=9)	90 $\pm$ 9 (n=9)	1.0 (n=13)	107 $\pm$ 11 (n=5)
STZ	0.11 $\pm$ 0.02 (n=9)	363 $\pm$ 15 (n=9)	0.97 $\pm$ 0.15 (n=13)	380 $\pm$ 14 (n=5)
STZ + INS	2.01 $\pm$ 0.30 (n=9) [†]	202 $\pm$ 35 (n=9) [†]	1.28 $\pm$ 0.28 (n=5) ^{NS}	181 $\pm$ 62 (n=5) [‡]

Animals received either a single intraperitoneal injection of normal saline (untreated controls) or STZ (125 mg per kg). After 3 days, the STZ-induced insulin-deficient rats were either left untreated or treated with insulin for 18 h before killing, as described in Fig. 1. Immediately before death, blood glucose values were again determined and are presented below. Northern blot analysis of total epididymal adipose tissue RNA (30 μ g) was performed as described in Fig. 1 using either the nick-translated GLUT-4 or GLUT-1 complementary DNA probes. The relative mRNA levels were determined by laser-scanning densitometry and normalized for the amount of glucose transporter mRNA present in the untreated control animals.

* Expression of the GLUT-4 and GLUT-1 glucose transporter mRNA relative to untreated controls.

[†] $P < 0.001$, [‡] $P < 0.01$ or NS (not significant) versus non-insulin-treated STZ-diabetic rats by unpaired *t*-test (two-tailed).

mRNA in rat adipose tissue. This decrease in GLUT-4 mRNA expression can be rapidly reversed by insulin treatment or refeeding. Although the expression of the GLUT-1 mRNA is regulated by serum, glucose, growth factors and transformation in various tissue-culture cells¹⁹⁻²³, our data demonstrate that adipose-tissue GLUT-1 mRNA levels are relatively unaffected by insulin deficiency *in vivo*. The altered expression of the insulin-responsive glucose transporter mRNA in diabetes and fasting *in vivo* directly correlates with the decrease in the insulin-response adipose glucose-transporter protein¹⁷ and inhibition of insulin-stimulated glucose transport activity¹⁴⁻¹⁶. Further studies are necessary to define the molecular basis for the metabolic control of GLUT-4 mRNA expression in insulin-responsive tissues as well as the potential for differential regulation of the other glucose-transporter mRNA species. □

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Control of transmembrane lipid asymmetry in chromaffin granules by an ATP-dependent protein

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THE Ca^{2+} -dependent binding of annexin proteins to secretory granule membranes seems to be involved in the early stage of exocytosis¹⁻³. Binding studies have shown that these proteins have a specificity for phosphatidylserine (PtdS) interfaces^{4,5}. Furthermore, aminolipids are necessary for contact and fusion between lipid vesicles⁶ or between liposomes and chromaffin granules⁷. Thus, PtdS must be present on the granule outer (cytoplasmic) monolayer. We report here that chromaffin granules possess a mechanism to maintain PtdS orientation, comparable to the ATP-dependent aminophospholipid translocase from human erythrocytes⁸⁻¹⁴. The translocase, in granules, selectively transports PtdS from the luminal to the cytoplasmic monolayer, provided the incubation medium contains ATP. As this protein shares several properties with the granule vanadate-sensitive ATPase II^{15,16}, we infer that this ATPase, of relative molecular mass 115,000, is the protein responsible for aminophospholipid translocation. This is the first evidence for an ATP-dependent specific phospholipid 'flippase' in intracellular organelles.

Using electron spin resonance (ESR), we have investigated the spontaneous reorientation, at 37 °C, of spin-labelled analogues of phosphatidylcholine (PtdC*) and phosphatidylserine (PtdS*) (Fig. 1) in chromaffin granules from bovine adrenal glands. Figure 2 shows the kinetics obtained with 3 mM Mg-ATP in the incubation medium. Previous studies^{8,14} have shown that in erythrocytes these spin-labelled lipids are faithful indicators of the endogenous lipid transmembrane distribution^{17,18}. By contrast with the situation in erythrocytes⁸⁻¹⁴, platelets¹⁹, lymphocytes²⁰ or fibroblasts²¹, where the outside-inside diffusion of aminophospholipids is faster than that of the choline-containing lipids, and also with liposomes, where all glycerophospholipids diffuse at the same rate²², the outside-inside

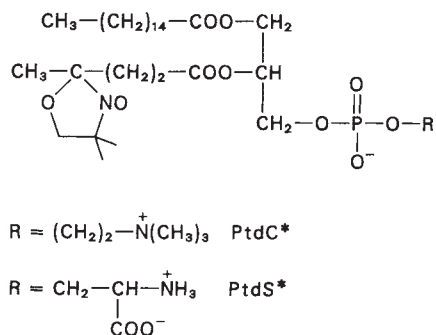


FIG. 1 Formula of the spin-labelled phospholipids used to investigate lipid transmembrane diffusion in chromaffin granules or inside-out erythrocytes. METHODS. The two analogues were synthesized as previously described^{8,14}. An amount of spin-label corresponding to 1% of the final amount of sample lipids was dried under vacuum, then resuspended in buffer before mixing with the membranes. Total incorporation was achieved within 1 min, as judged from the ESR spectrum. Transmembrane distribution of probes was determined at any time by a 1-min incubation of a sample-aliquot with defatted bovine serum albumin (BSA) followed by centrifugation and recording of the ESR spectrum of the BSA-containing supernatant with a Varian E-9 spectrometer operating at 9.15 GHz. The buffer containing BSA had to be supplemented with 10 mM K^+ hexaferriyanide to reoxidize the reduced nitroxides¹⁴.

diffusion of PtdS* in granules is slower than that of PtdC*. The equilibrium distribution corresponds to ~85% of PtdS* and 60% of PtdC* on the cytoplasmic face. If Mg-ATP is not added and its formation from leaky granules is prevented by addition of EDTA, then PtdC* translocation is not affected, but PtdC* flips like PtdS*, that is, diffusion is accelerated and reaches a lower plateau. If, after 150 min, the medium is supplemented with an excess of Mg-ATP (see Fig. 2), PtdS* returns to a higher level corresponding to more PtdS* on the cytoplasmic face with a half-time of inside-outside diffusion of ~20 min. Conversely, when EDTA was added after 150 min to Mg-ATP-containing medium, the slow decrease of the percentage of outwardly exposed PtdS* resumed, but did not reach the plateau of PtdS* after an additional hour (data not shown). These results suggest that with ATP in the external medium, PtdS* is translocated back to the cytosolic face. It seems therefore that the transmembrane lipid asymmetry in chromaffin granules is due to a specific carrier inverted with respect to the aminophospholipid translocase of plasma membranes.

By comparison, we have investigated lipid translocation in inside-out erythrocyte vesicles (Fig. 3). Without Mg-ATP in the incubation medium, PtdS* and PtdC* showed identical kinetics, leading to an equilibrium with ~50-60% of the probe on the cytoplasmic leaflet. The presence of Mg-ATP did not modify

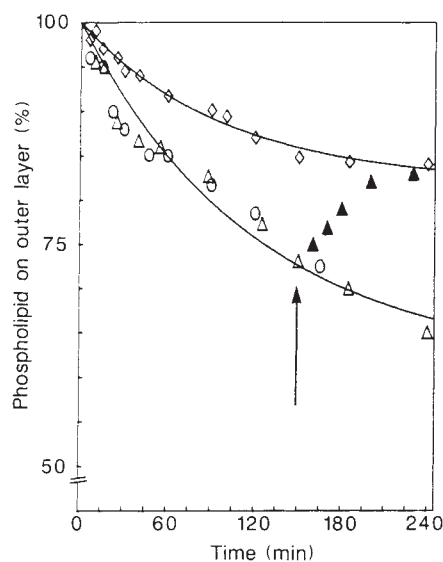


FIG. 2 Transmembrane redistribution at 37 °C of PtdS* and PtdC* initially incorporated on the outer surface of chromaffin granules: (○) PtdC* and (◇) PtdS* with 3 mM Mg-ATP in the incubation medium; (△) PtdS with 1 mM EDTA in the incubation medium. At the point indicated by an arrow, an aliquot of the sample initially incubated with 1 mM EDTA was supplemented with 4 mM Mg-ATP and the kinetics of PtdS* reorientation followed (▲).

METHODS. Bovine chromaffin granules were prepared from bovine adrenal medulla by homogenization followed by differential centrifugation and isopycnic separation on Percoll-gradient²⁹. This procedure led to a mitochondria-free fraction. Incubation conditions were as follows: 1 ml purified chromaffin granules (3 mM phospholipid) in 0.3 M sucrose-HEPES buffer (10 mM, pH 7.3) was diluted with 1 ml of the same buffer supplemented either with 9 mM Mg-ATP, 20 mM creatine phosphate and creatine phosphokinase (100 IU ml⁻¹) or with 3 mM EDTA. After a 5-min pre-incubation at 37 °C, the reaction was initiated by addition of 1 ml of pre-heated suspension of PtdS* or PtdC* (30 μM) in buffer. At given intervals, aliquots (120 μl) were taken and mixed with 60 μl of 3% (w/v) BSA in buffer. After 1 min on ice, the mixture was centrifuged (2 min 7,600g) and the supernatant assayed by ESR. The intensity of the central peak gave the percentage of spin-labels on the outer monolayer. After 4 h at 37 °C, 10% lysis could be detected as evaluated by the release of soluble proteins; consequently the kinetics were not pursued. The curves shown here, as well as in Fig. 3, are fitted to an exponential function by least-square, nonlinear regression.

the reorientation rate of PtdC*, although PtdS* did behave differently and tended to a more asymmetrical distribution (77% outside). This plateau was lower than the plateau obtained in erythrocytes^{8,14}; the inside-out vesicles however, are contaminated by right-side out vesicles²³, which should decrease the equilibrium level. Thus, at least qualitatively, Fig. 3 confirms the interpretation of Fig. 2: chromaffin granules are naturally occurring 'inside-out' vesicles provided with a specific translocase.

To ascertain the involvement of a protein in PtdS* translocation, the granules were incubated with N-ethylmaleimide (NEM) for 10 min at 4 °C, before the addition of PtdS* and Mg-ATP. NEM at a concentration of 25 μ M, which inhibits granule ATPase I (the proton pump)^{15,16,24} had no effect on PtdS* translocation; on the other hand, 2 mM NEM had the same effect as Mg-ATP depletion, PtdS* followed PtdC* kinetics. A millimolar concentration of NEM is required for inhibition of the aminophospholipid translocase in red cells¹⁰ and for inhibition of the vanadate-sensitive Mg-ATPase II from granules^{15,16,24}. Because ATPase I and ATPase II are the only ATPases detected in chromaffin granules, we infer that ATPase II, whose function is unknown, is the aminophospholipid translocase. This hypothesis is supported by the fact that ATPase II and the aminophospholipid translocase form an acylphosphate intermediate²⁴. ATPase II has a relative molecular mass of ~115,000, as determined by phosphorylation²⁴ and purification¹⁶.

Although the precise mechanism of granule exocytosis is not known, conditions favouring membrane fusion are well documented, especially the importance of amino-phospholipids in the adjacent membranes as recalled in the first paragraph¹⁻⁷. Fusion of chromaffin granules to the plasma membrane of chromaffin cells, although mediated by Ca^{2+} , requires Mg^{2+} and hydrolysable ATP, the substrates of the aminophospholipid translocase²⁵. We suggest therefore that the translocase, whose existence in a cell organelle is shown here for the first time, is important at least to make the granules fusion-competent and, hence, is one of the proteins necessary for exocytosis. ATPase activities, sharing the properties of ATPase II, have been

described in other organelles which interact with the plasma membrane, namely cholinergic synaptic vesicles²⁶ and coated vesicles²⁷; thus one might also expect to find an aminophospholipid translocase activity in such membranes. On the other hand, the microsomal flippase^{13,28} does not seem to create lipid asymmetry. Finally, a mitochondria-rich fraction from bovine adrenal medulla revealed no specific aminolipid translocation (data not shown). The presence of an aminophospholipid translocase may thus be restricted to organelles involved in fusion processes. □

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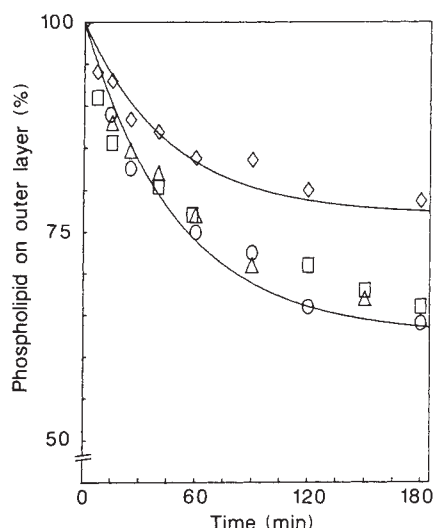


FIG. 3 Transmembrane redistribution at 37 °C of PtdS* and PtdC* initially incorporated in the outer monolayer of inside-out erythrocyte vesicles: (◇) PtdS* with Mg-ATP; (△) PtdS* without Mg-ATP; (○) PtdC* with Mg-ATP; (□) PtdC* without Mg-ATP.

METHODS. The protocol for the kinetics was the same as that used for chromaffin granules, except that the buffer was 150 mM NaCl, 0.1 mM EGTA, 10 mM Tris, pH 7.4. Fresh blood from healthy volunteers was obtained from a blood bank (Fondation Nationale de Transfusion Sanguine). Inside-out vesicles were prepared from washed human erythrocytes by the method of Sarkadi *et al.*²³ and used within a day.

Interleukins 4 and 5 control expression of IL-2 receptor on murine B cells through independent induction of its two chains

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RESTING B cells express few, if any, receptors for interleukin-2 (IL-2), whereas activated B cells can express receptors for and respond to IL-2 (refs 1, 2). IL-2 receptors can exist on the cell surface in three different forms; the complete high-affinity receptor, a heterodimer consisting of a chain of relative molecular mass (M_r) 70-75,000 (70-75K) and a chain of M_r 55K; the 70-75K chain alone, with intermediate affinity for IL-2; or the 55K chain alone, with low affinity for IL-2 (ref. 3). We have previously reported that IL-5-stimulated B cells are induced to express the 55K chain^{1,4}. We report here evidence for the differential regulation of the expression of the two chains, namely that IL-4 and IL-5 can independently induce expression of the 70-75K and 55K chains respectively on murine B cells. As expected, cells stimulated to

express the 55K chain alone are unresponsive to IL-2, whereas cells stimulated to express either the 70-75K chain or the 70-75/55K heterodimer respond to IL-2, at a high and low ligand concentration respectively, with a marked increase in proliferation. This orchestration of receptor expression and factor responsiveness may represent a novel activation pathway for B cells, where the two chains of a compound receptor are shown to be independently regulated.

The activation of B lymphocytes to antibody formation usually involves a multiplicity of signals. One activation pathway that has been extensively investigated is that involving the synergistic action of so-called T-cell-independent antigens and one or more interleukins⁵⁻⁸. This has documented that at least six cytokines, namely IL-1, IL-2, IL-4, IL-5, IL-6 and interferon- γ , singly or in various combinations, can influence the process. Although it is frequently argued that these factors act sequentially, for example IL-4 promoting early activation steps, IL-5 growth and IL-6 terminal differentiation, study of single B cells developing into antibody-forming clones⁸ shows the reality to be more complex, with different cytokines having multiple and overlapping roles. It was therefore of interest to determine to what degree regulation of expression of the various receptors for cytokines may aid in understanding this activation pathway.

We have reported previously¹ that IL-2 receptors can be induced on murine splenocytes enriched for B cells by the action of growth and differentiation factors derived from EL4-thymoma cell line conditioned medium (EL4-BGDF). Furthermore, EL4-BGDF-stimulated B cells proliferate in response to IL-2. We also noted that stimulation with IL-5, but not with IL-1, IL-2,

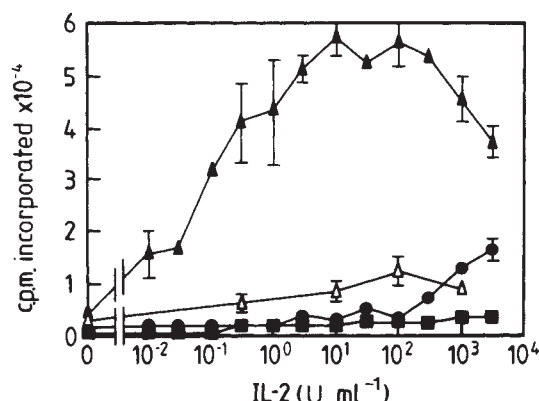


FIG. 1 Response of stimulated B cells to IL-2. The proliferative response to IL-2 of B cells, pre-cultured in RPMI 1640 medium with 5% fetal calf serum alone (■), or medium supplemented with purified recombinant murine IL-4 at 1,000 units ml^{-1} (●); purified recombinant murine IL-5 at 1% (△); or both IL-4 and IL-5 (▲) at the above concentrations, was investigated using [^3H]TdR uptake¹.

METHODS. CBA/CAH/WEHI splenocytes enriched for B cells by the use of a complement cell-lysis step using antibodies against T cells and macrophages were cultured for 3 days in medium supplemented as described above. They were then collected, washed and recultured in triplicate in the presence of various concentrations of highly-purified recombinant, human IL-2 (a gift from Cetus). After 24 h, 1 μCi [^3H]TdR was added to each well and 16 h later wells were harvested and counted for β -emissions. Data from a single representative experiment are presented as the mean counts $\pm$ s.d. (vertical bars) per min (c.p.m.) incorporated from triplicate wells. Where no error bars are shown, the s.d. was within the limits of the symbol. The maximal ratio of IL-2 responsiveness was calculated by dividing the number of c.p.m. incorporated when cells were recultured in the presence of an optimal concentration of IL-2 by the number of c.p.m. incorporated when cells were recultured in medium alone. For cells initially cultured in medium alone this ratio was 2; with IL-4, 140; with IL-5, 4; and with IL-4 + IL-5, 15. This experiment was repeated several times with similar results. Purified recombinant murine IL-5 was a gift from Dr C. Sanderson²⁴. A concentration of 1% was just maximal for both proliferation and immunoglobulin production by B cells²⁵. Purified recombinant murine IL-4 was a gift from Immunex²⁶.

IL-3, or IL-4, could induce the expression of the 55K chain of the IL-2 receptor. We therefore investigated whether IL-5-stimulated cells could respond to IL-2 with proliferation. Surprisingly, only a minimal response was observed (Fig. 1). This suggested that some activity in EL4-BGDF other than IL-5 was required for the induction of fully functional IL-2 receptors and thus of IL-2 responsiveness. We tested several cloned lymphokines for a capacity to synergise with IL-5 in inducing IL-2 responsiveness; of the factors tested, only IL-4 had this activity (Fig. 1). Cells stimulated with IL-4 alone proliferated in response to IL-2 with an unusual response curve. Instead of the half-maximal response at ~ 1 unit ml^{-1} of IL-2 seen with EL4-BGDF-stimulated B cells, IL-4-stimulated cells showed a half-maximal response at ~ 100 units ml^{-1} IL-2. This response curve was similar to that noted by Wang and Smith⁹ for a natural killer-cell line and by Tanaka *et al.*¹⁰ for a human B-lymphoblastoid line which expressed only the medium affinity 70-75K chain of the IL-2 receptor. As the 70-75K chain alone and the 70-75/55K heterodimer can transduce a biological signal upon ligation by IL-2, whereas the low-affinity 55K chain by itself is incapable of transducing a proliferative signal¹¹, it seemed possible that IL-5-stimulated cells express only the 55K chain (the only chain recognized by available monoclonal antibodies) and that this could explain their unresponsiveness to IL-2. IL-4, on the other hand, might induce expression of the medium affinity 70-75K chain, which would explain the shift in the IL-2 response curve noted with IL-4-stimulated cells. This shift was only noted at concentrations $\geq 1,000$ units ml^{-1} of IL-4, a threshold similar to that required for IgE isotype switching by B cells¹². This concentration of IL-4 is much higher than that required to induce B cells to express Ia antigens, to proliferate in response to anti-immunoglobulin or to secrete IgG1 antibodies^{5,6}.

To test this hypothesis, we assayed IL-4-stimulated B cells for expression of the 55K chain using fluorescence-activated cell sorter (FACS) analysis of cells stained with the anti-IL-2 receptor monoclonal antibody PC61 (ref. 13 and Table 1). Whereas IL-5 or EL4-BGDF-stimulated cells bound this antibody, IL-4-stimulated cells did not. In addition, whereas IL-5- and EL4-BGDF-stimulated B cells release a soluble form of the 55K chain as determined by an enzyme-linked immunosorbent assay (ELISA) using anti-55K chain antibodies⁴, IL-4 did not have this effect (Table 1). These findings suggest that IL-4 stimulation does not induce either expression of the 55K chain by B cells or its shedding from their surface, whereas IL-5 does both⁴.

To address directly the question of whether IL-4 leads to the expression of the 70-75K chain of the receptor, we performed

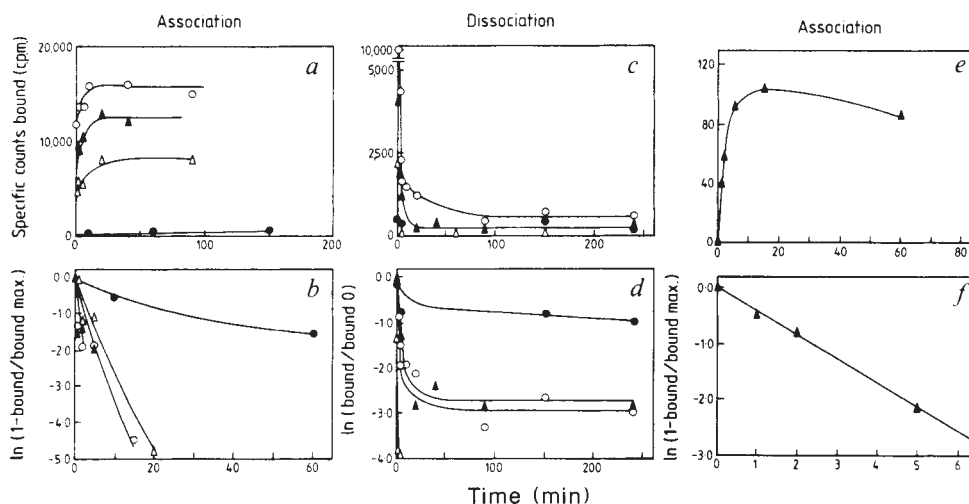
TABLE 1 Cell-surface expression of the 55K chain and the release of soluble IL-2 receptors (55K chains) by stimulated B cells

Cell stimulation	% PC61 (55K chain) positive	Soluble IL-2R (55K chain) (pg ml^{-1})
Medium alone	<2	<10
IL-4 (1,000 units ml^{-1})	<2	<10
IL-5 (1%)	40	300

Splenocytes enriched for B cells as described in Fig. 1 were cultured with or without purified recombinant murine IL-4 or IL-5. Determination of cell surface expression of the 55K chain of the IL-2 receptor was performed using the antibody PC61 known to recognize an epitope on the 55K chain of the murine IL-2 receptor with subsequent FACS analysis as previously described¹. The release of the 55K chain of the IL-2 receptor by cells after 5 days in culture was measured using an ELISA specific for the receptor⁴. Values given relate to single experiments and represent the mean of 3 separate wells for soluble IL-2 receptor release and a single determination for cell-surface expression of the receptor. These experiments were repeated with similar results.

FIG. 2 Association (panels *a*, *b*, *e* and *f*) and dissociation (panels *c* and *d*) kinetic binding data for CTLL cells (○) and IL-4 (●), IL-5 (△) and IL-4 + IL-5-stimulated (▲) splenocytes.

METHODS. Splenocytes from 6–8 week-old Balb/c nu/nu mice were cultured for 3 days in 250 ml flasks with 2.5×10^6 cells ml^{-1} in the presence of 3,000 units ml^{-1} of recombinant murine IL-4 and/or 10 units ml^{-1} of recombinant murine IL-5. After 3 days, cells were washed and resuspended in RPMI supplemented with 5% FCS. CTLL cells are an IL-2-dependent T-cell line known to express both high- and low-affinity receptors for IL-2. Cells were harvested 3 days after their last passage, washed and resuspended in KDS/RPMI supplemented with 5% FCS. For association studies either [^{125}I]IL-2 (Amersham) alone, to a final concentration of 2 nM (panels *a* and *b*) or 80 pM (panels *e* and *f*), or with a 500-fold excess of unlabelled recombinant human IL-2 (Cetus) was added to the cell suspension on ice for varying periods from 20 s to 1.5 h. Mainly high-affinity receptors should bind IL-2 at 80 pM, which is a concentration 125-fold lower than the K_D of the low-affinity receptor. IL-5-stimulated cells did not specifically bind [^{125}I]IL-2 at this concentration (data not shown). The reaction volume was then overlaid onto 180 μl FCS in a tube and centrifuged at 8,000 r.p.m. in a biofuge Eppendorf centrifuge for 2 min to separate bound from unbound counts. The cell pellet was then cut off and the pellet and remainder of the tube counted separately in a crystal multidetector radioimmunoassay system (United Technologies Packard). The c.p.m. in the cell pellet reacted with an excess of unlabelled IL-2 was subtracted from the c.p.m. in the cell pellet reacted with [^{125}I]IL-2 alone, to determine the number of specific counts bound. For dissociation studies (panels *c* and *d*) cells were reacted either with [^{125}I]IL-2 at 2 nM alone or in the presence of a 500-fold excess of unlabelled IL-2 for 4 h and then 50 μl of the resuspended reaction volume was overlaid onto 180 μl FCS in a tube and centrifuged at



8,000 r.p.m. for 2 min. The cell pellet was then cut off and cells resuspended in KDS/RPMI supplemented with 5% FCS and unlabelled IL-2 to a final concentration of 1 μM . After being reacted on ice in this volume for from 20 s to 4 h the reaction volume was collected and overlaid on 180 μl FCS in a tube. This tube was centrifuged and the cell pellet and cell supernatant collected as described above. The cell pellet and cell supernatant were counted in a liquid scintillation counter for γ -emissions and the amount of specific counts bound to the cell pellet and those dissociated during the reaction calculated for each time point. Each point represents the mean of two experimental points from a single experiment. Data are presented as time versus specific counts bound (panels *a*, *c* and *e*), and as time versus a natural log transformation of the binding data (panels *b*, *d* and *f*). Experiments were repeated with similar results. Recombinant murine IL-5 was supernatant from FD cells (a cloned, factor-dependent, mast cell-like line) transfected with a Zen vector containing the gene²⁷. Recombinant murine IL-4 was supernatant from monkey COS cells transfected with an SV40 vector containing the gene²⁸.

kinetic binding studies using radioiodinated IL-2. It has been demonstrated using various cell lines that all three forms of the IL-2 receptor, that is the 70–75/55K heterodimer, the 70–75K and the 55K chains, have characteristic association and dissociation equilibrium and kinetic binding constants at 4 °C (refs 9, 14). The 70–75/55K heterodimer has a fast association and a

slow dissociation and a high affinity; the 70–75K chain has both slow association and dissociation and a medium affinity; and the 55K chain has both fast association and dissociation kinetic binding constants and a low affinity for IL-2. The differences in the kinetic binding constants are striking and readily measurable.

TABLE 2 Association and dissociation kinetic binding and equilibrium constants for stimulated splenocytes

	Kinetic binding constants		Association k $\text{M}^{-1} \text{s}^{-1}$	Calculated equilibrium dissociation constants (K_D) k'/k M	Determined equilibrium dissociation constants K_D M
	Dissociation k' (s^{-1})	$t_{1/2}$			
Splenocytes + IL-4(70–75K)*	7.7×10^{-5}	150 min	1.6×10^{-4}	4.8×10^{-9}	3.2×10^{-10}
+ IL-5(55K)	3.4×10^{-2}	20 s	1.0×10^6	3.4×10^{-8}	ND
+ IL-4/5(55K)	2.5×10^{-2}	28 s	8.9×10^6	2.8×10^{-9}	3.3×10^{-9}
(70–75/55K)	8.7×10^{-5}	133 min	8.0×10^7	1.1×10^{-12}	2.4×10^{-12}
CTLL(55K)	2.5×10^{-2}	28 s	8.5×10^6	2.9×10^{-9}	5.4×10^{-9}
(70–75/55K)	2.8×10^{-4}	42 min	8.0×10^7	3.5×10^{-12}	1.6×10^{-12}

* Form of the IL-2 receptor postulated to be expressed by these cells.

ND, not done.

The association and dissociation kinetic, and dissociation equilibrium, constants were calculated from natural log plots of binding data⁹. The slope of the dissociation plot is $-k'$ and that of the association plot is $k' + k[H]$, where $[H]$ is the concentration of radiolabelled ligand. The half time for dissociation is $0.693/(1/k')$. Kinetic association and dissociation experiments were repeated with similar results. The two dissociation values for CTLL cells and IL-4 + IL-5-stimulated cells reflect the bimodal nature of binding to these cells which is due to the existence of two classes of receptors on their surface. In addition to the calculated equilibrium dissociation constants, these were also determined in further work from the negative inverse of the slope of Scatchard plots of specific binding data. Binding was performed at 4 °C under conditions similar to those for kinetic binding experiments; 3×10^5 CTLL cells or 3×10^6 nude mouse splenocytes precultured for 3 days with IL-4 or IL-4 + IL-5, as described for Fig. 2, were harvested and then reacted with various concentrations of [^{125}I]IL-2 with or without a 500-fold excess of unlabelled IL-2. Bound and unbound counts were similarly separated and counted as for Fig. 2. Scatchard plots of binding curves of specific counts bound showed good linearity. With B cells + IL-4, Scatchard plots showed only one receptor population. Plots from data on CTLL cells and IL-4 + IL-5-stimulated B cells clearly showed high- and low-affinity receptor populations with a marked excess of the latter.

As large numbers of B cells were required for binding studies, cells from the spleens of 6–8-week-old BALB/c nu/nu mice, which did not contain any T cells (as assessed by FACS analysis using antibodies against several T-cell markers) were used to avoid the cell losses associated with the B-cell enrichment procedure. Stimulation of these cells with IL-4, IL-5, IL-4+IL-5 or EL4-BGDF and subsequent assay for IL-2 responsiveness and IL-2 receptor expression revealed results similar to those in Fig. 1. Association and dissociation kinetic and equilibrium binding constants of induced IL-2 receptors for ^{125}I -labelled IL-2 were determined (Fig. 2; Table 2) for various stimulated B-cell populations. These displayed the features characteristic of all three forms of the receptor. Whereas B cells examined immediately after preparation or cultured in medium alone did not specifically bind ^{125}I -labelled IL-2, IL-4-stimulated cells bound ^{125}I -labelled IL-2 in a manner characteristic of expression of the 70–75K chain of the IL-2 receptor alone; IL-5-stimulated cells in a manner characteristic of expression of the 55K chain alone; and IL-4+IL-5-stimulated cells and the positive control CTLL cells (an IL-2-dependent cloned T cell line) in a manner characteristic of expression of both 55K chains and the 70–75/55K heterodimer. The expression of very low numbers of 70–75K or 55K chains by IL-5- or IL-4-stimulated cells respectively cannot, however, be excluded. Our derived equilibrium dissociation constants (K_D) are not far from those reported previously by Wang and Smith⁹: 4.8×10^{-9} versus 1.2×10^{-9} for the 70–75K chain; 3.4×10^{-8} versus 1.4×10^{-8} for the 55K chain; and 1.1×10^{-12} versus 1.3×10^{-11} for the 70–75/55K heterodimer. Although the values for the 70–75/55K heterodimer differ somewhat, the values for the experimental (IL-4+IL-5-stimulated cells) and the positive control (CTLL cells) groups were very similar. This data also revealed that IL-4+IL-5- and IL-4-stimulated cells expressed similar numbers of 70–75/55K high-affinity and 70–75K medium-affinity receptors respectively (Fig. 2c). The average number of these receptors per cell was ~5–10% of the average number of 70–75/55K receptors on the CTLL cells.

Autograph study of B cells cultured with 1,000 units ml^{-1} of IL-4 and then reacted with 3nM ^{125}I -labelled IL-2 with or without a 500-fold excess of unlabelled IL-2 revealed specific binding of ^{125}I -labelled IL-2 to ~20% of cells. In this and similar studies, however, the exact proportion of cells of which receptors were induced showed considerable variation. Scatchard analysis of equilibrium binding data (Table 2) revealed high- and low-affinity receptors on IL-4+IL-5-stimulated and CTLL cells, and medium-affinity IL-2 receptors on IL-4-stimulated cells. At low concentrations (pM), no specific binding of IL-2 to IL-5-stimulated B cells could be detected. The numbers of receptors on other experimental groups were much lower than those for CTLL cells. Because of the heterogeneity in labelling, the exact number on the induced subset will have to be the subject of further study.

In contrast to the situation with T cells, IL-2 has been thought of as only a weakly active B-cell factor^{15–17}. This is in spite of the now well documented existence of both high- and low-affinity IL-2 receptors on appropriately stimulated B cells^{15,18}. In light of our present findings, this disparity between the action of IL-2 on T and B cells may reflect the specific requirements of B cells in order to express functional high-affinity IL-2 receptors. When B cells do receive these specific stimuli, namely IL-4+IL-5, they respond to maximal IL-2 stimulation with a marked increase in tritiated thymidine (^3H]TdR) uptake (Fig. 1). As cells precultured in medium alone do not respond to IL-2 with increased proliferation, but display a relatively high level of IL-2-independent proliferation, it appears that IL-4+IL-5 induces IL-2-dependence for proliferation.

This activation and receptor expression pathway depends on lymphokines alone, unaided by antigen or mitogens, and is probably confined to only a subset of B cells. As our present studies have been restricted to bulk cultures, the frequency of responding cells has not yet been determined. Given the sig-

nificant percentage of receptor-positive cells after culture, however, and the appreciable IL-2-dependent proliferation (Fig. 1), a considerable number of B cells have probably been stimulated. This novel activation pathway of B cells, depending on IL-4+IL-5+IL-2, is separate from that involving antigen or anti-Ig+IL-4, or from stimulation by lipopolysaccharide. The degree to which the triple synergy between lymphokines facilitates antibody production is the subject of current study, as is the possible role of other B-cell-active cytokines such as interferon- γ and IL-6 in modifying B-cell responses, with particular regard to isotype expression. It is already apparent that B cells stimulated with IL-4+IL-5+IL-2 alone do not achieve maximal antibody production and that further signals are needed, a finding consistent with a recent report¹⁹ that anti-Ig-treated B-cell blasts stimulated with IL-4 and IL-5 show an increase in IgG1 production when IL-2 is also added.

There are several examples where one cytokine regulates the expression of the receptor for another cytokine. These include up-regulation of IL-2 receptors on T cells by IL-1^{20,21}, and the ability of IL-3 to down-regulate the expression of other cytokine receptors on haemopoietic colony-forming cells^{22,23}. To our knowledge, this is the first example of a situation where the two chains of a single receptor are separately regulated by two different cytokines to permit a co-ordinated appearance of functional high-affinity receptors. This subtlety of regulation permits a novel and sophisticated level of control. Although the function of this dual regulation is not clear, it is known that IL-4 exerts a powerful influence early in B-cell activation. If it alone induced the full IL-2 receptor, a B cell might be driven down an IL-4+IL-2 differentiation pathway prematurely or inappropriately, whereas if IL-5, with its own capacity to induce growth and differentiation, is also required, the effects of the later-acting IL-2 would thereby be constrained. A tight and complex regulation of receptor expression might also limit unwanted 'bystander' effects in antibody responses, thus helping to maintain the specificity of an immune response. As more lymphokine receptors become amenable to study, it will be of interest to extend the present investigation to see if their expression is also subject to unexpectedly complex control mechanisms. □

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Industrial involvement in education

Richard Pearson

Demographics and a harsh economic climate are changing the way governments approach higher education. Finance and other support are being sought from the private sector.

THROUGHOUT the world higher education is in the throes of major changes. With a near universal decline in the numbers of young people the search is on for new entrants. At the same time rising costs are leading governments to look to the private sector for additional funding and support.

In the United Kingdom the government is also stressing the need for industrialists to be members of the governing bodies of educational institutions at all levels from the primary to higher education, as well as of national advisory bodies and the newly formed funding councils in higher education. This is intended both to increase the relevance of higher education to the needs of the economy and to provide an injection of private sector management skills, while the pressure is also there to provide additional resources.

Training needs

These links reflect a broader demand for the involvement of industrialists in activities that were once regarded as the prerogative of the state. Another example is the development, by the government, of the national network of Training and Enterprise Councils (TECs) as the latest solution to Britain's training problem. This programme is underpinned by the belief that training needs can only be identified by the employers at a local level and that the skills of businessmen rather than civil servants are needed to develop and manage the training programmes.

In these and many other areas the demands on employers' time and resources are growing; will they be willing and able to respond? While some industrialists are responding on altruistic grounds, many others are becoming involved because of their increasing concern about shortages of appropriately skilled people leaving the education system at all levels and the likely skill shortages in the next decade. Many companies argue, however, that it is not their business to be involved in this way, but rather to make profits and leave the taxation system to be used to meet national objectives.

In the case of higher education the traditional linkages through recruitment, student sponsorship and the funding of research are being extended to improve the image of the employer so as to reinforce its place in the recruitment market and its potential to exploit new research

findings. This is coming about through more donations of finance and equipment, staff time for seminars and lectures, work placements for students and advice on a wide range of activities from the curricula to careers services.

As yet, however, only a minority of companies appear to be doing this in any concerted way with most building up links on an *ad hoc* basis. The more developed companies that are becoming involved are starting to appoint campus liaison managers whereby an existing, usually senior, member of staff coordinates, but does not direct, the contacts that build up between the company and a series of individual campuses. The aim is to ensure that these contacts reinforce each other and that, for example, research links are known about on the recruitment side and that good students have a positive image of the company and can possibly be encouraged to join it by the professor or academic supervisor. In a broader-based move BP is proposing a fund of £3 million aimed at boosting participation in higher education. Development projects in areas close to BP operations will seek to boost low age participation rates through activities such as training for admission tutors and the development and coordination of access courses involving both schools and higher education.

As yet the pattern and motivation of employer involvement in the fast-moving world of education is uncharted but some lessons may be available from the rise in corporate involvement in local job-creation initiatives. This followed the recession of the early 1980s when economic decline led to the rise of a plethora of special initiatives to improve the plight of communities with particularly high levels of unemployment. In parallel with the government's emphasis on small firms came the rise in corporate responsibility with firms becoming involved with economic and job-creation activities outside their normal business. They did so for a wide range of reasons (see *The Charitable Role of Companies*, IMS, 1989). Some were prompted by their own redundancies, others by peer and political pressure, others by the belief that a successful economy was good for their long-term business prosperity, while a few became involved for reasons of altruism. Involvement was largely initiated and sustained by the interest of a key board member, in contrast to a lot of educational involve-

ment which has sprung up at the level of the operating unit. In the case of job creation support was principally directed at those areas where there would be a pay back for the company through a more positive image and an improved local environment.

Some companies have job-creation budgets in excess of £1 million, while other similar companies have budgets of under £50,000. This support involves money and resources, secondments and staff time. Few companies, however, have clear objectives or targets for their activities or monitor their effectiveness, and in many cases these activities are in direct competition with educational and training activities for a share of the company's charitable budget. Because support is usually directed to areas where they have a local presence, many deprived localities, who by definition often lack the presence of big business locally, as well as ethnic groups and others such as the long-term unemployed, often miss out.

Adjusting priorities

The demands on corporate responsibility are growing rapidly. In this climate of change there will be a growing need for companies to clarify and target their objectives more precisely if their support is to have the maximum benefit. For higher education the need will be to spell out the benefits more clearly to the giver who, especially in education, will be expecting a clear pay back. This may in turn lead to pressure on higher education to adjust its programmes and priorities, whether in research or teaching, to the needs of a small group of corporate givers. We already see that in the case of student sponsorship there are courses which, by design, are filled with sponsored students. As such the curriculum often reflects the needs of these, usually large, companies, and as the majority of students join their sponsoring company on graduation it means that small and new recruiters have little or no realistic access to some of the best students who are on some of the 'best' courses in the country. The challenge for higher education, then, is to be able to take on board the benefits of corporate involvement, of which there are many, without distorting the basic purpose of education itself. □

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